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ATGTCAAGCCTTTTACGTGAATTGTATGCTAAACCCTTATCAGAACGCCATGTAGAATCTGATGGCC
TTATTTTCGACCCAGCGCAAATCACAAGTCGAACCGCCAATGGTGTTGCTGTACCGCACGGAGACC
ATTATCACTTTATTCCTTATTCACAACTGTCACCTTTGGAAGAAAAATTGGCTCGTATTATTCCCCTTC
GTTATCGTTCAAACCATTGGGTACCAGATTCAAGACCAGAACAACCAAGTCCACAATCGACTCCGG
AACCTAGTCCAAGTCCGCAACCTGCACCAAATCCTCAACCAGCTCCAAGCAATCCAATTGATGAGA
AATTGGTCAAAGAAGCTGTTCGAAAAGTAGGCGATGGTTATGTCTTTGAGGAGAATGGAGTTCCTC
GTTATATCCCAGCCAAGGATCTTTCAGCAGAAACAGCAGCAGGCATTGATAGCAAACTGGCCAAGC
AGGAAAGTTTATCTCATAAGCTGCAGTTAGATCCATTA

(57) Abstract

The present invention relates to the identification and use of a family of human complement C3–degrading polypeptides expressed by *S.pneumoniae*. The polypeptides have molecular weights of about 15 kD to about 25 kD or about 75 kDa to about 95 kDa, for example. Preferred polypeptides of this invention include the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:5.

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HUMAN COMPLEMENT C3-DEGRADING POLYPEPTIDE FROM *STREPTOCOCCUS PNEUMONIAE*

Field of the Invention

This invention relates to *Streptococcus pneumoniae* and in particular this invention relates to the identification of an *S. pneumoniae* polypeptide that is capable of degrading human complement protein C3.

Background of the Invention

Respiratory infection with the bacterium *Streptococcus pneumoniae* (*S. pneumoniae*) leads to an estimated 500,000 cases of pneumonia and 47,000 deaths annually. Those persons at highest risk of bacteremic pneumococcal infection are infants under two years of age, individuals with a compromised immune system and the elderly. In these populations, *S. pneumoniae* is the leading cause of bacterial pneumonia and meningitis. Moreover, *S. pneumoniae* is the major bacterial cause of ear infections in children of all ages. Both children and the elderly share defects in the synthesis of protective antibodies to pneumococcal capsular polysaccharide after either bacterial colonization, local or systemic infection, or vaccination with purified polysaccharides. *S. pneumoniae* is the leading cause of invasive bacterial respiratory disease in both adults and children with HIV infection and produces hematogenous infection in these patients (Connor et al. *Current Topics in AIDS* 1987;1:185-209 and Janoff et al. *Ann. Intern. Med.* 1992;117(4):314-324).

Individuals who demonstrate the greatest risk for severe infection are not able to make antibodies to the current capsular polysaccharide vaccines. As a result, there are now four conjugate vaccines in clinical trial. Conjugate vaccines consist of pneumococcal capsular polysaccharides coupled to protein carriers or adjuvants in an attempt to boost the antibody response. However, there are other potential problems with conjugate vaccines currently in clinical trials. For example, pneumococcal serotypes that are most prevalent in the United States are different from the serotypes that are most common in places

such as Israel, Western Europe, South Africa, or Scandinavia. Therefore, vaccines that may be useful in one geographic locale may not be useful in another. The potential need to modify currently available capsular polysaccharide vaccines or to develop protein conjugates for capsular vaccines to suit geographic serotype variability entails prohibitive financial and technical complications. Thus, the search for immunogenic, surface-exposed proteins that are conserved worldwide among a variety of virulent serotypes is of prime importance to the prevention of pneumococcal infection and to the formulation of broadly protective pneumococcal vaccines. Moreover, the emergence of penicillin and cephalosporin-resistant pneumococci on a worldwide basis makes the need for effective vaccines even more exigent (Baquero et al. *J. Antimicrob. Chemother.* 1991;28S;31-8).

Several pneumococcal proteins have been proposed for conjugation to pneumococcal capsular polysaccharide or as single immunogens to stimulate immunity against *S. pneumoniae*. Surface proteins that are reported to be involved in adhesion of *S. pneumoniae* to epithelial cells of the respiratory tract include PsaA, PspC/CBP112, and IgA1 proteinase (Sampson et al. *Infect. Immun.* 1994;62:319-324, Sheffield et al. *Microb. Pathogen.* 1992; 13: 261-9, and Wani, et al. *Infect. Immun.* 1996; 64:3967-3974). Antibodies to these adhesins could inhibit binding of pneumococci to respiratory epithelial cells and thereby reduce colonization. Other cytosolic pneumococcal proteins such as pneumolysin, autolysin, neuraminidase, or hyaluronidase are proposed as vaccine antigens because antibodies could potentially block the toxic effects of these proteins in patients infected with *S. pneumoniae*. However, these proteins are typically not located on the surface of *S. pneumoniae*, rather they are secreted or released from the bacterium as the cells lyse and die (Lee et al. *Vaccine* 1994; 12:875-8 and Berry et al. *Infect. Immun.* 1994; 62:1101-1108). While use of these cytosolic proteins as immunogens might ameliorate late consequences of *S. pneumoniae* infection, antibodies to these proteins would neither promote pneumococcal death nor prevent initial or subsequent pneumococcal colonization.

A prototypic surface protein that is being tested as a pneumococcal

vaccine is the pneumococcal surface protein A (PspA). PspA is a heterogeneous protein of about 70-140 kDa. The PspA structure includes an alpha helix at the amino terminus, followed by a proline-rich sequence, and terminates in a series of 11 choline-binding repeats at the carboxy-terminus. Although much information regarding its structure is available, PspA is not structurally conserved among a variety of pneumococcal serotypes, and its function is entirely unknown (Yother et al. *J. Bacteriol.* 1992;174:601-9 and Yother *J. Bacteriol.* 1994;176:2976-2985). Studies have confirmed the immunogenicity of PspA in animals (McDaniel et al. *Microb. Pathogen.* 1994; 17;323-337). Despite the immunogenicity of PspA, the heterogeneity of PspA, its existence in four structural groups (or clades), and its uncharacterized function complicate its ability to be used as a vaccine antigen.

In patients who cannot make protective antibodies to the type-specific polysaccharide capsule, the third component of complement, C3, and the associated proteins of the alternative complement pathway constitute the first line of host defense against *S. pneumoniae* infection. Because complement proteins cannot penetrate the rigid cell wall of *S. pneumoniae,* deposition of opsonic C3b on the pneumococcal surface is the principal mediator of pneumococcal clearance. Interactions of pneumococci with plasma C3 are known to occur during pneumococcal bacteremia, when the covalent binding of C3b, the opsonically active fragment of C3, initiates phagocytic recognition and ingestion (Johnston et al. *J. Exp. Med* 1969;129:1275-1290, Hasin HE, *J. Immunol.* 1972; 109:26-31 and Hostetter et al. *J. Infect. Dis.* 1984; 150:653-61). C3b deposits on the pneumococcal capsule, as well as on the cell wall. This method for controlling *S. pneumoniae* infection is fairly inefficient. Methods for augmenting *S. pneumoniae* opsonization could improve the disease course induced by this organism. There currently exists a strong need for methods and therapies to limit *S. pneumoniae* infection.

Summary of the Invention

This invention relates to the identification and use of a family of human

complement C3-degrading polypeptides (e.g., proteins) expressed by *S. pneumoniae*. The polypeptides preferably have a molecular weight of about 15 kDa to about 25 kDa, or about 75 kDa to about 95 kDa, as determined, for example, on a 10% SDS polyacrylamide gel. The invention includes a number of polypeptides isolatable from different C3-degrading strains of *S. pneumoniae*.

In one aspect, the invention relates to an isolated polypeptide having at least 80% sequence identity with SEQ ID NO:2 or SEQ ID NO:5. In a preferred embodiment, the polypeptide is isolated from *S. pneumoniae* or alternatively the polypeptide is a recombinant polypeptide. Preferably, the isolated polypeptide degrades human complement protein C3. A preferred polypeptide of this invention is an isolated polypeptide having an amino acid sequence that includes SEQ ID NO:2 or SEQ ID NO:5, and more preferably, is SEQ ID NO:2 or SEQ ID NO:5. The term "isolated" as used herein refers to a naturally occurring species that has been removed from its natural environment, as well as to synthetic species. The term "polypeptide" as used herein includes peptides, polypeptides, and proteins, regardless of length. Preferably, the polypeptides of the invention include one or more functional units, which encompasses polypeptides that degrade human complement protein C3.

Thus, the invention also relates to polypeptide fragments isolated from a C3-degrading protein of this invention. Such fragments are encompassed by the term "polypeptide" as used herein. Preferably, the invention provides polypeptides of at least 15 sequential amino acids derived from a protein that has at least 80% sequence identity with SEQ ID NO:2 or SEQ ID NO:5, and more preferably, polypeptides of at least 15 sequential amino acids of SEQ ID NO:2 or SEQ ID NO:5. In another aspect of this invention, preferred polypeptides are capable of degrading human complement protein C3.

In another aspect, the invention relates to an isolated polypeptide that preferably degrades human complement protein C3, wherein nucleic acid encoding the isolated polypeptide hybridizes to at least a portion of SEQ ID NO:1 or SEQ ID NO:4 or their complementary strands under highly stringent hybridization conditions. Preferably, the polypeptide includes at least 15

sequential amino acids, which are, more preferably, of SEQ ID NO:2 or SEQ ID NO:5.

In an additional aspect, this invention relates to polypeptides that have reduced human C3 degradation activity or which do not degrade human C3; however, the nucleic acids encoding this group of polypeptides each include a nucleotide sequence that hybridizes to either the nucleic acids that encode human C3 degrading polypeptides, or the complementary strands for each nucleic acid. This latter group of polypeptides having reduced or no human C3 degrading activity are referred to herein as "non-degrading" polypeptides. The non-degrading polypeptides may differ from C3 degrading polypeptides by one or more amino acids. This amino acid change may be a substitution, alteration, or deletion of one or more amino acids. Various types of amino acid changes are discussed herein. Nucleic acids encoding the non-degrading polypeptides are alternatively preferred embodiments of this invention.

The invention also relates to an immune system stimulating composition (preferably, a vaccine) comprising an effective amount of an immune system stimulating polypeptide of the present invention, which is preferably isolated from *S. pneumoniae*, and a therapeutically acceptable carrier. In one embodiment, the immune system stimulating composition or vaccine further comprises at least one other immune system stimulating polypeptide isolated from *S. pneumoniae*.

The invention further relates to an antibody capable of binding (typically, specifically binding) to a polypeptide (at least a portion thereof) of the present invention. In one embodiment, the antibody is a monoclonal antibody and in another embodiment, the antibody is a polyclonal antibody. In another embodiment the antibody is an antibody fragment. The antibody or antibody fragments can be obtained from a mouse, a rat, a goat, a chicken, a human, or a rabbit.

The invention also relates to an isolated nucleic acid molecule (i.e., a polynucleotide, which can be single stranded or double stranded, and which can be a part, or fragment, of a larger molecule such as a vector) capable of hybridizing to at least a portion of SEQ ID NO:1 or SEQ ID NO:4 or their

complimentary strands under highly stringent hybridization conditions. As used herein, highly stringent hybridization conditions include, for example, 6XSSC, 5X Denhardt, 0.5% SDS, and 100 μg/ml fragmented and denatured salmon sperm DNA hybridized overnight at 65°C and washed in 2X SSC, 0.1% SDS one time at room temperature for about 10 minutes followed by one time at 65°C for about 15 minutes followed by at least one wash in 0.2XSSC, 0.1% SDS at room temperature for at least 3-5 minutes. In one embodiment, the nucleic acid molecule is isolated from *S. pneumoniae* and in another embodiment, the nucleic acid molecule encodes a polypeptide. In one embodiment, the polypeptide degrades human complement protein C3. In another embodiment, the nucleic acid molecule encodes a polypeptide that does not degrade human complement C3.

In another embodiment, the nucleic acid molecule is in a vector (i.e., it is a fragment of a nucleic acid vector). The vector can be an expression vector capable of producing a polypeptide. Cells containing the nucleic acid molecule are also contemplated in this invention. In one embodiment, the cell is a bacterium or a eukaryotic cell.

The invention further relates to an isolated nucleic acid molecule comprising the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:4, or their complementary strands. The invention further relates to an RNA molecule transcribed by a double-stranded DNA sequence comprising SEQ ID NO:1 or SEQ ID NO:4.

In another aspect of this invention, the invention relates to a method for producing an immune response to *S. pneumoniae* in a mammal (particularly a human). The method includes: administering to a mammal a composition comprising a therapeutically effective amount of a polypeptide of the present invention, and a pharmaceutically acceptable carrier, to produce an immune response to the polypeptide. The immune response can be a B cell response, a T cell response, an epithelial response, or an endothelial response. In a preferred embodiment, the composition is a vaccine composition. Preferably the polypeptide is at least 15 amino acids in length and also preferably the composition further comprises at least one other immune system stimulating

polypeptide from *S. pneumoniae*. In one embodiment, the polypeptide comprises at least 15 amino acids of SEQ ID NO:2 or SEQ ID NO: 5.

The invention further relates to an isolated polypeptide of about 15 kDa to about 25 kDa, or about 75 kDa to about 95 kDa, from *Streptococcus pneumoniae* that is capable of degrading human complement C3 and to a method for inhibiting *Streptococcus pneumoniae*-mediated C3 degradation. The method includes contacting a *Streptococcus pneumoniae* bacterium with antibody capable of binding to a polypeptide (at least a portion thereof) of the present invention.

The invention also relates to a method for inhibiting C3-mediated inflammation and rejection in xenotransplantation. The method includes expressing on the surface of an organ of an animal used in xenotransplantation a polypeptide of the present invention. This method is particularly advantageous for causing, for example, the kidneys of pigs to express the polypeptide described herein and thereby to inhibit C3 mediated inflammation after xenotransplantation.

The invention also relates to an isolated nucleic acid molecule that contains a region of at least 15 nucleotides which hybridize under highly stringent hybridization conditions to at least a portion of a nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:4 or their complementary strands.

The invention also relates to isolated DNA molecules or primers having the nucleic acid sequences as shown in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 AND SEQ ID NO:9.

Brief Description of the Figures

Figure 1 provides the nucleic acid sequence of the translated portion of a C3-degrading polypeptide (approximately 20 kDa) gene of this invention (SEQ ID NO:1).

Figure 2 provides the amino acid sequence of a C3-degrading polypeptide (approximately 20 kDa) of this invention (SEQ ID NO:2).

Figure 3 diagrams the amino acid sequence of a C3-degrading polypeptide (approximately 20 kDa) - sitioned with the nucleic acid sequence

(double stranded) encoding a C3-degrading polypeptide according to this invention (SEQ ID NOS:1-3 wherein SEQ ID NO:3 is the complement of SEQ ID NO:1).

Figure 4 provides the nucleic acid sequence for a predicted 92 kDa amino acid sequence (SEQ ID NO:4).

Figure 5 provides the predicted 92 kDa amino acid sequence (SEQ ID NO:5).

Figure 6 shows sequence alignments of SEQ ID NO:1 and a portion of SEQ ID NO:4.

Figure 7 shows sequence alignment of SEQ ID NO:2 with a portion of SEQ ID NO:5.

Figure 8. Western blot analysis of several pneumococcal whole cell lysate with polyclonal anti—r20 kDa sera. Molecular weight markers were run in lane 1; recombinant 20 kDa polypeptide from pDF122 was run in lane 2; recombinant 92 kDa polypeptide from Type 7 was run in lane 3; lane 4 was a blank; CP1200 whole cell lysate was run in lane 5; Type 3 whole cell lysate was run in lane 6; and Type 7 whole cell lysate was run in lane 7. The antisera recognizes the recombinant polypeptides of approximately 20 kDa and approximately 92 kDa, but only recognized the larger polypeptide in whole cell lysates.

Figure 9. Autoradiogram showing degradation of biotinylated C3 by 20 kDa and 92 kDa polypeptides of the present invention.

Figure 10. Coomassie Blue-Stained 7.5% SDS-PAGE analysis of C3 degradation by the 20 kDa (Lane A8) and the 92 kDa (Lane C8) polypeptides of the present invention.

Figure 11 is a chart which shows the survival of CBA/CAHN *xid*/J mice immunized SC (subcutaneous) with r79 kDa protein adjuvanted with MPL and challenged IN (intranasal) with *S. pneumoniae* Type 3.

Figure 12 is a SDS-PAGE gel described in Example 8.

Detailed Description of the Preferred Embodiments

The present invention relates to the identification and isolation of a human complement C3 degrading polypeptide fragment of a larger polypeptide of about 75 kD to about 95 kD. This fragment has a molecular weight of about 20 kDa (± 5 kDa) on a 10% SDS-PAGE gel. It also relates to nucleic acids encoding C3 degrading polypeptides.

It has been observed that exponentially growing cultures of pneumococci from several serotypes were able to first degrade the β-chain then degrade the α chain of C3 without producing defined C3 cleavage fragments (Angel, et al. *J. Infect. Dis.* 170:600-608, 1994). This pattern of degradation without cleavage differs substantially from that of other microbial products such as the elastase enzyme of *Pseudomonas aeruginosa* and the cysteine proteinase of *Entamoeba histolytica.*

The term "degrade" is used herein to refer to the removal of amino acids from proteinaceous molecules, generating peptides or polypeptides. The polypeptide of this invention degrade C3 without generating the cleavage fragments known as C3b, iC3b, or C3d. There is at least some preference of the C3-degrading polypeptides of this invention for C3 in that, for example, the C3-degrading polypeptide does not appear to degrade other proteins, such as albumin.

A C3-degrading polypeptide of about 20 kDa was isolated from a library of insertionally interrupted pneumococcal genes by identifying those clones that had decreased C3 degrading activity as compared to wild type *S. pneumoniae*.An exemplary assay for assessing C3-degrading activity of clones is provided in Example 1. Clones with decreased C3-degrading activity were identified and a 546 bp SmaI insert was selected, based on the sequence of the clones that had demonstrated decreased C3-degrading activity. This SmaI fragment was used to probe an *S. pneumoniae* library made from strain CP1200. Positive clones from the *S. pneumoniae* library that hybridized to the SmaI fragment were isolated and the open reading frame of the gene associated with C3-degrading activity was identified. The following oligonucleotide (SEQ ID NO:10), which has sequence identify with a portion of PspA, was used to confirm, by differential

hybridization, that the gene encoding the C3-degrading protinease was distinct from the gene encoding PspA.

SEQ ID NO:10

GAAAACAATAATGTAGAAGACTACTTTAAAGAAGGTTAGA

An open reading frame of a 20 kDa polypeptide spans an area of approximately 500 base pairs (SEQ ID NO:1) predicting a polypeptide of molecular weight of about 20 kDa (+/- 5 kDa) or about 168 amino acids (SEQ ID NO:2). An exemplary gene sequence encoding a C3-degrading polypeptide is provided in Figure 1 as SEQ ID NO:1 and an amino acid sequence of the polypeptide is provided in Figure 2 as SEQ ID NO:2. Figure 3 combines a preferred gene sequence with a corresponding preferred translated polypeptide as SEQ ID NOS:1-3.

Using SEQ ID NO:2, the amino acid sequence of the approximately 20 kDa polypeptide, was determined to be unrelated to other polypeptides in the GenBank or Swiss Prot databases. The predicted polypeptide encompasses a proline-rich sequence characteristic of membrane domains in prokaryotes, particularly between amino acids 80-108 suggesting that the polypeptide is expressed at the surface. The amino acid sequence exhibits no apparent choline-binding repeats. Electrophoresis of pneumococcal lysates and supernatants from cultures of CP 1200 on SDS-PAGE gels impregnated with C3 identified a lytic band at about 20 kDa (± 5 kDa) in both supernatants and lysates, confirming that a polypeptide of a size predicted by SEQ ID NO:2 had C3-degrading activity (see Example 2). As provided in Example 3, the gene encoding the 20 kDa C3-degrading polypeptide is conserved in at least two dozen pneumococcal isolates representing five serotypes (serotypes 1, 3, 4, 14, and 19F).

The nucleotide sequence encoding the C3-degrading polypeptide of this invention was inserted into a gene expression vector for expression in *E. coli*. Recombinant C3-degrading polypeptide was isolated as described in the examples. Those of ordinary skill in the art recognize that, given a particular gene sequence such as that provided in SEQ ID NO:1, there are a variety of

expression vectors that could be used to express the gene. Further, there are a variety of methods known in the art that could be used to produce and isolate the recombinant polypeptide of this invention and those of ordinary skill in the art also recognize that the C3 degrading assay of this invention will determine whether or not a particular expression system, in addition to those expression systems provided in the examples, is functioning, without requiring undue experimentation. A variety of molecular and immunological techniques can be found in basic technical texts such as those of Sambrook et al. (*Molecular Cloning, A Laboratory Manual*, 1989 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) and Harlow et al. (*Antibodies; A Laboratory Manual.* Cold Spring Harbor, NY; Cold Spring Harbor Laboratory Press, 1988).

The polynucleotide encoding the C3 degrading polypeptide of this invention was identified using a plasmid library made with pneumococcal genomic DNA fragments from strain CP1200. Although there are a variety of methods known for obtaining a plasmid library, in a preferred strategy, a plasmid library was constructed with Sau 3A digested pneumococcal genomic DNA fragments (0.5 -4.0 kb) from pneumococcal strain CP 1200 (obtained from D.A. Morrison, University of Illinois, Champagne-Urbana, Illinois and described in Havarstein LF, et al. *Proc. Natl. Acad. Sci. (USA)* 1995;92:11140-11144) and inserted into the Bam HI site of the integrative shuttle vector pVA 891 (erm^r, cm^r; has origin of replication for *E. coli*). This library was transformed into an *E. coli* DH5α MCR strain by electroporation. Plasmid extractions of some randomly selected *E. coli* transformants revealed that all of them contained recombinant plasmids.

Plasmid library DNA can be extracted from the *E. coli* transformants and used to transform the CP 1200 parent pneumococcal strain using insertional mutagenesis by homologous recombination.

The pneumococcal strain CP 1200 cells can be made competent using a pH shift with HCl procedure in CTM medium. The competent cells are frozen at -70°C in small aliquots until needed.

The isolated polypeptide of this invention can be incubated with human complement C3 for 4 hours or longer at 37°C in the presence of PBS to detect

C3 degradation. Control samples without the isolated pneumococcal polypeptide are used as controls for comparative purposes.

The polypeptides of this invention have an apparent molecular weight on a 10% SDS-polyacrylamide gel of either about 15 kDa to about 25 kDa; and preferably about 20 kDa; or about 75 kDa to about 95 kDa; and preferably about 92 kDa. Exemplary polypeptides sequences are provided by SEQ ID NO: 2 and SEQ ID NO:5. Those of ordinary skill in the art will recognize that some variability in amino acid sequence is expected and that this variability should not detract from the scope of this invention. For example, conserved mutations do not detract from this invention nor do variations in amino acid sequence identity of less than about 80% amino acid sequence identity and preferably less than about 90% amino acid sequence identity where the polypeptide is capable of degrading human complement protein C3, and particularly where the polypeptide is isolated or originally obtained from an *S. pneumoniae* bacterium. Proteins and fragments thereof (all referred to as polypeptides) are also within the scope of the present invention, particularly if they are capable of degrading human complement protein C3.

Some nucleic acid sequence variability is expected among pneumococcal strains and serotypes as is some amino acid variability. Conserved amino acid substitutions are known in the art and include, for example, amino acid substitutions using other members from the same class to which the amino acid belongs. For example, the nonpolar amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, and tryptophan. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such alterations are not expected to affect apparent molecular weight as determined by polyacrylamide gel electrophoresis or isoelectric point. Particularly preferred conservative substitutions include, but are not limited to, Lys for Arg and *vice verse* to maintain a positive charge; Glu for Asp and vice versa to maintain a negative charge; Ser for Thr so that a free -OH is maintained; and Gln for Asn to maintain a free NH_2.

Preferred polypeptides of this invention includes polypeptides with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5. Other polypeptides include those degrading human complement polypeptide C3 and having nucleic acid encoding the polypeptide that hybridizes to SEQ ID NO:1 or SEQ ID NO:4 under highly stringent hybridization conditions such as 6XSSC, 5X Denhardt, 0.5% SDS (sodium dodecyl sulfate), and 100 µg/ml fragmented and denatured salmon sperm DNA hybridized overnight at 65°C and washed in 2X SSC, 0.1% SDS one time at room temperature for about 10 minutes followed by one time at, 65°C for about 15 minutes followed by at least one wash in 0.2XSSC, 0.1% SDS at room temperature for at least 3-5 minutes are also contemplated in this invention. Typically, an SSC solution contains sodium chloride, sodium citrate, and water to prepare a stock solution.

The polypeptides of this invention can be isolated or prepared as recombinant polypeptides. That is, nucleic acid encoding a protein, or a portion thereof, can be incorporated into an expression vector or incorporated into a chromosome of a cell to express the polypeptide in the cell. The polypeptide can be purified from a bacterium or another cell, preferably a eukaryotic cell and more preferably an animal cell. Alternatively, the polypeptide can be isolated from a cell expressing the polypeptide, such as a *S. pneumoniae* cell. Thus, proteins, peptides, or polypeptides are all considered within the scope of this invention when the term "polypeptide" is used. The polypeptides are preferably at least 15 amino acids in length and preferred polypeptides have at least 15 sequential amino acids from SEQ ID NO:2 or SEQ ID NO:5.

Nucleic acid encoding the about 15 kDa to about 20 kDa polypeptide and the about 75 kD to about 95 kD polypeptide are also part of this invention. SEQ ID NOS:1 and 4 are preferred nucleic acid molecules encoding a C3-degrading polypeptide. Those of ordinary skill in the art will recognize that some substitution will not alter the C3-degrading polypeptide sequence to an extent that the character or nature of the C3-degrading polypeptide is substantially altered. For example, nucleic acid with an identity of at least 80% to SEQ ID NO:1 is contemplated in this invention. A method for determining whether a particular nucleic acid sequence falls within the scope of this invention is to

consider whether or not a particular nucleic acid sequence encodes a C3-degrading polypeptide and has a nucleic acid identity of at least 80% as compared with SEQ ID NO:1 or SEQ ID NO:4. Other nucleic acid sequences encoding the C3 polypeptide include nucleic acid encoding the C3 polypeptide where the C3 polypeptide has the same sequence or at least a 90% sequence identity with SEQ ID NO:2 or SEQ ID NO:5 but which includes degeneracy with respect to the nucleic acid sequence. A degenerate codon means that a different three letter codon is used to specify the same amino acid. For example, it is well known in the art that the following RNA codons (and therefore, the corresponding DNA codons, with a T substituted for a U) can be used interchangeably to code for each specific amino acid:

Phenylalanine (Phe or F)	UUU, UUC, UUA or UUG
Leucine (Leu or L)	CUU, CUC, CUA or CUG
Isoleucine (Ile or I)	AUU, AUC or AUA
Methionine (Met or M)	AUG
Valine (Val or V)	GUU, GUC, GUA, GUG
Serine (Ser or S)	AGU or AGC
Proline (Pro or P)	CCU, CCC, CCA, CCG
Threonine (Thr or T)	ACU, ACC, ACA, ACG
Alanine (Ala or A)	GCU, GCG, GCA, GCC
Tryptophan (Trp)	UGG
Tyrosine (Tyr or Y)	UAU or UAC
Histidine (His or H)	CAU or CAC
Glutamine (Gln or Q)	CAA or CAG
Asparagine (Asn or N)	AAU or AAC
Lysine (Lys or K)	AAA or AAG
Aspartic Acid (Asp or D)	GAU or GAC
Glutamic Acid (Glu or E)	GAA or GAG
Cysteine (Cys or C)	UGU or UGC
Arginine (Arg or R)	AGA or AGG
Glycine (Gly or G)	GGU or GGC or GGA or GGG
Termination codon	UAA, UAG or UGA

Further, a particular DNA sequence can be modified to employ the codons preferred for a particular cell type. For example, the preferred codon usage for *E. coli* is known, as are preferred codons for animals including humans. These changes are known to those of ordinary skill in the art and therefore these gene sequences are considered part of this invention. Other nucleic acid sequences include at least 15, and preferably, at least 30 nucleic acids in length from SEQ ID NO:1 or SEQ ID NO:4 or other nucleic acid fragments of at least 15, and preferably at least 30 nucleic acids in length where these fragments hybridize to SEQ ID NO:1 or SEQ ID NO:4 under highly stringent hybridization conditions such as 6XSSC, 5X Denhardt, 0.5% SDS, and 100 μg/ml fragmented and denatured salmon sperm DNA hybridized overnight at 65°C and washed in 2X SSC, 0.1% SDS one time at room temperature for about 10 minutes followed by one time at, 65°C for about 15 minutes followed by at least one wash in 0.2XSSC, 0.1% SDS at room temperature for at least 3-5 minutes.

The nucleic acid molecules of this invention can encode all, none (i.e., fragments that cannot be transcribed, fragments that include regulatory portions of the gene, or the like), or a portion of SEQ ID NO:2 or SEQ ID NO:5 and preferably containing a contiguous nucleic acid fragment that encodes at least nine amino acids from SEQ ID NO:2 or SEQ ID NO:5. Because nucleic acid molecues encoding a portion of a C3 degrading polypeptide are contemplated in this invention, it will be understood that not all of the nucleic acid molecules will encode a protein or peptide or polypeptide with C3 degrading activity. Further, the nucleic acid of this invention can be mutated to remove or otherwise inactivate the C3 degrading activity of this polypeptide. Therefore, nucleic acid molecules that encode polypeptides without C3 degrading activity that meet the hybridization requirements described above are also contemplated. Methods for mutating or otherwise altering nucleic acid sequences are well described in the art and the production of an immunogenic, but enzymatically inactive polypeptide can be tested for therapeutic utility.

The nucleic acid molecules of this invention can be incorporated into nucleic acid vectors or stably incorporated into host genomes to produce

recombinant polypeptides including recombinant chimeric polypeptides. In one embodiment, the C3-degrading polypeptide is encoded by a gene in a vector and the vector is in a cell. Preferably, the cell is a prokaryotic cell such as a bacterium. The genes and gene fragments can exist as the fusion of all or a portion of the gene with another gene and the C3-degrading polypeptide can exist as a fusion protein of one or more proteins where the fusion protein is expressed as a single protein. A variety of nucleic acid vectors of this invention are known in the art and include a number of commercially available expression plasmids or viral vectors. The use of these vectors is well within the scope of what is ordinary skill in the art. Exemplary vectors are employed in the examples, but should not be construed as limiting on the scope of this invention.

This invention also relates to antibodies capable of binding (typically specifically binding) to polypeptides of about 15 kDa to about 25 kDa; and preferably about 20 kDa; and about 75 kDa to about 95 kDa; and preferably about 92 kDa, from *S. pneumoniae* and preferably where the polypeptides are capable of degrading human complement C3. Polyclonal or monoclonal antibodies can be prepared to all or part of the polypeptides of the invention Methods for preparing antibodies to polypeptides are well known and well described, for example, by Harlow et al., (*Antibodies; A Laboratory Manual.* Cold Spring Harbor, NY; Cold Spring Harbor Laboratory Press, 1988). In a preferred example, the antibodies can be human derived, rat derived, mouse derived, goat derived, chicken derived, or rabbit derived. Polypeptide-binding antibody fragments and chimeric fragments are also known and are within the scope of this invention.

The invention also relates to the use of immune stimulating compositions. The term "immune stimulating" or "immune system stimulating" composition refers to protein, peptide, or polypeptide compositions according to the invention that activate at least one cell type of the immune system in a subject, such as a mammal. Preferably, the immune stimulating composition provides an immunizing response or prophylactic benefit in a normal, i.e., uninfected subject, typically a vaccine. However, any measurable immune response is beneficial to the subject in a therapy application or protocol.

Preferred activated cells of the immune system include phagocytic cells such as neutrophils or macrophages, T cells, B cells, epithelial cells and endothelial cells. Immune stimulating compositions comprising the peptides, polypeptides or proteins of the invention can be used to produce antibody in an animal such as a rat, mouse, goat, chicken, rabbit, or a human or an animal model for studying *S. pneumoniae* infection. Preferred immune stimulating compositions include an immune stimulating amount, e.g, a therapeutically effective amount, of at least one peptide or polypeptide including at least 15 amino acids from the C3 degrading polypeptide.

The term "vaccine" refers to a composition for immunization. This process can include the administration of a protein, peptide, polypeptide, antigen, nucleic acid sequence or complementary sequence, e.g., anti-sense, or antibody, or suspensions thereof, wherein upon administration, the molecule will produce active immunity and provide protection against an *S. pneumoniae* infection or colonization. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The vaccine preparation may optionally be emulsified, or encapsulated in liposomes.

The immune stimulating composition (such as a vaccine) can further include other polypeptides in a pharmaceutically acceptable buffer or carrier, such as PBS (phosphate buffer saline) or another buffer recognized in the art as suitable and safe for introduction of polypeptides into a host to stimulate the immune system. The immune stimulating compositions can also include other immune system stimulating polypeptides such as adjuvants or immune stimulating proteins, peptides, or polypeptides from *S. pneumoniae* or other organisms. For example, a cocktail of peptides or polypeptides may be most useful for controlling *S. pneumoniae* infection. Preferably one or more of the polypeptides, or fragments thereof, of this invention are used in a vaccine preparation to protect against or limit *S. pneumoniae* colonization or the pathogenic consequences of *S. pneumoniae* colonization.

A "therapeutically effective amount," as used herein, refers to that amount that is effective for production of a desired result. This amount varies

depending upon the health and physical condition of a subject's immune system, i.e., to synthesize antibodies, the degree of protection desired, the formulation prepared and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

The active immune stimulating ingredients are often mixed with excipients or diluents that are pharmaceutically acceptable as carriers and compatible with the active ingredient. The term "pharmaceutically acceptable carrier" refers to a carrier(s) that is "acceptable" in the sense of being compatible with the other ingredients of a composition and not deleterious to the recipient thereof. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the immune stimulating composition (including vaccine) may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the immune stimulating compostion.

Examples of adjuvants or carriers that may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL + TDM + CWS) in a 2% squalene/Tween 80 emulsion.

This invention also relates to a method for inhibiting *Streptococcus pneumoniae*-mediated C3 degradation comprising contacting a *Streptococcus pneumonie* bacterium with a polypeptide, such as an antibody or another polypeptide that is capable of binding to an isolated polypeptide (typically, at least a portion thereof) of about 15 kDa to about 25 kDa, or about 75 kDa to about 95 kDa, from *Streptococcus pneumoniae*. The protein capable of binding to an isolated polypeptide of about 15 kDa to about 25 kDa, or about 75 kDa to about 95 kDa can be an antibody or a fragment thereof, or the protein can be a

chimeric protein that includes the antibody binding domain, such as a variable domain, from antibody that is capable of specifically recognizing an isolated polypeptide of about 15 kDa to about 25 kDa, or about 75 kDa to about 95 kDa, from *Streptococcus pneumoniae* having C3 degrading activity.

The isolated *S. pneumoniae* polypeptide of this invention can be isolated, and optionally purified, and the isolated polypeptide or immunogenic fragments thereof can be used to produce an immunologic response, including, in one example, an antibody response in a human or an experimental animal. Polypeptides without C3 degrading ability can be tested for their ability to limit the effects of *S. pneumoniae* infection. Similarly, the polypeptides of this invention can be modified, such as through mutation to interrupt or inactivate the C3 degrading activity of the polypeptides. Antibody capable of inhibiting the C3-degrading activity of the polypeptides of this invention may be used as a strategy for preventing C3 degradation and for promoting clearance of *S. pneumoniae* through the opsonic pathway. Isolated polypeptides can be used in assays to detect antibody to *S. pneumoniae* or as part of a vaccine or a multi-valent or multiple protein, peptide, or polypeptide-containing vaccine for *S. pneumoniae* therapy.

Thus, the term "treatment," as used herein, refers to prophylaxis and/or therapy of either normal mammalian subjects or mammalian subjects colonized with, diagnosed with, or exhibiting characteristics or symptoms of various *S. pneumoniae* infections. The term "therapy" refers to providing a therapeutic effect to a mammalian subject such that the subject exhibits few or no symptoms of a pneumococcal infection or other related disease. Such treatment can be accomplished by administration of nucleic acid molecules (sense or antisense), proteins, peptides or polypeptides or antibodies of the instant invention.

It is further contemplated that the polypeptides of this invention can be surface expressed on vertebrate cells and used to degrade C3, for example, where complement deposition (or activation) becomes a problem, such as in xenotransplantation or in complement-mediated glomerulonephritis. For example, the entire pneumococcal polypeptide, a recombinant polypeptide, or a portion of either, can be incorporated into xenotransplant cells and expressed as

a surface polypeptide or as a secreted polypeptide to prevent or minimize complement deposition (and/or complement-mediated inflammation).

Another specific aspect of the present invention relates to using a vaccine vector expressing an isolated protein, and peptides or polypeptides therefrom. Accordingly, in a further aspect this invention provides a method of inducing an immune response in a mammal, which comprises providing to a mammal a vaccine vector expressing at least one, or a mixture of an isolated protein and/or peptide or polypeptide of the invention. The protein and peptides or polypeptides of the present invention can be delivered to the mammal using a live vaccine vector, in particular using live recombinant bacteria, viruses or other live agents, containing the genetic material necessary for the expression of the protein and/or peptides or polypeptides as a foreign polypeptide. Particularly, bacteria that colonizes the gastrointestinal tract, such as Salmonella, Shigella, Yersinia, Vibrio, Escherichia and BCG have been developed as vaccine vectors, and these and other examples are discuessed by J. Holmgren et al., Immunobiol., 184, 157-179 (1992) and J. McGhee et al., Vaccine, 10, 75-88 (1992).

An additional embodiment of the present invention relates to a method of inducing an immune response in a subject, e.g,. mammal, comprising administering to the subject an amount of a DNA molecule encoding an isolated protein and/or peptide or polypeptide therefrom of this invention, optionally with a transfection-facilitating agent, where the protein and/or peptides or polypeptides retain immunogenicity and, when incorporated into an immune stimulating composition, e.g, vaccine, and administered to a human, provides protection without inducing enhanced disease upon subsequent infection of the human with *S. pneumoniae* pathogen. Transfection-facilitating agents are known in the art.

It is further contemplated that the antisense sequence of the gene encoding the about 15 kDa to about 25 kDa polypeptide, and the about 75 kDa to about 95 kDa polypeptide may be used as a vaccine or as a therapeutic treatment for pneumococcal infection. Antisense DNA is defined as a non-coding sequence that is complementary, i.e., a complementary strand, to all or a portion of SEQ ID NO:1 or SEQ ID NO:4. For example, the antisense sequence for 5'-

ATGTCAAGC-3' is 3'-TACAGTTCG-5'. Delivery of antisense sequence or oligonuclotides into an animal may result in the production of antibody by the animal or in the incorporation of the sequence into living bacteria or other cells whereby transcription and/or translation of all or a portion of the 92 kDa gene product is inhibited.

Introduction of an antisense nucleic acid sequence can be accomplished, for example, by loading the antisense nucleic acid into a suitable carrier, such as a liposome, for introduction into pneumococci or infected cells. Typically, an antisense nucleic acid sequence having eight or more nucleotides is capable of binding to the bacterial nucleic acid or bacterial messenger RNA. The antisense nucleic acid sequence, typically contains at least about 15 nucleotides, preferably at least about 30 nucleotides or more nucleotides to provide necessary stability of a hybridization product of bacterial nucleic acid or bacterial messenger RNA. Introduction of the sequences preferably inhibit the transcription or translation of at least one endongenous *S. pneumoniae* nucleic acid sequence. Methods for loading antisense nucleic acid is known in the art as exemplified by U.S. Patent 4,242,046.

The present invention also provides nucleic acid having an open reading frame of 2478 bases (SEQ ID NO:4) that encompasses the open reading frame of a nucleic acid sequence (SEQ ID NO:1) that encodes a polypeptide that has a molecular weight of about 20 kDa (SEQ ID NO:2). The 20 kDa polypeptide, described herein, is further characterized as a C3-degrading polypeptide. The larger open reading frame, e.g., 2163 bp (SEQ ID NO:4), encodes for a putative polypeptide of about 92 kDa (SEQ ID NO:5).

All references and publications cited herein are expressly incorporated by reference into this disclosure. There are a variety of alternative techniques and procedures available to those of skill in the art which would similarly permit one to successfully perform the intended invention in view of the present disclosure. It will be appreciated by those skilled in the art that while the invention has been described above in connection with particular embodiments and examples, the invention is not necessarily so limited and that numerous other embodiments, examples, uses, modifications and departures from the embodiments, examples

and uses may be made without departing from the inventive scope of this application.

Example 1

Identification of Insertional Mutants with Reduced C3-Degrading Activity

Insertional mutants were received from Dr. Elaine Tuomanen, (Rockefeller Inst., New York, New York). The clones with insertions were tested in an assay to detect reduced C3-degrading activity. 137 clones were tested by growing the cells in Todd Hewitt broth overnight at room temperature in microtitre plates. The cells were diluted 1:10 in synthetic medium for pneumococci (see Sicard A. M., *Genetics* 50:31-44, 1984) and the remainder of the cells were frozen in the microtiter plate. Either 63 ng or 83 ng of C3 (purified from human plasma according to the method of Tack et al., *Meth. Enzymol.* 80:64-101, 1984) per 100 µl of medium containing 1 mg/ml of 0.1% BSA in phosphate buffered saline (PBS) was added to about 200 µl of diluted cells. The cells were incubated at 37°C for 4 hrs. One hundred µl of the mixture was added to ELISA plates and incubated overnight at 4°C. The plates were washed three times with wash buffer and the wells were filled with 0.05% Tween 20 in PBS with five minute incubations between the washes. One hundred µl of antibody to C3 (polyclonal horse-radish peroxidase-conjugated goat antibody specific to human C3-IgG fraction, ICN Cappel, Costa Mesa, CA) was diluted 1:1200 with 3% BSA in PBS. The ELISA plate was incubated at 37°C for about 30 minutes to 1 hr in the dark and washed with wash buffer as above. The assay was developed using 12 mg of OPD in 30 ml of 0.1M sodium citrate buffer with 12 µl of 30% hydrogen peroxide. Assay results were determined by optical density readings at 490 nm on an ELISA plate reader.

Each clone was tested four times. Nineteen clones were selected that had less than 40% C3 degradation as compared to nonmutated controls. These 19 clones were screened 6 times by the assay described above and from these results 5 clones were selected with less than 30% C3-degrading activity as

compared to controls. These 6 clones were screened eleven times each and the two clones with the lowest C3-degrading activity were selected for further study.

A partial sequence of one of the clones was received and a *SmaI* fragment of 546 bp was labeled with ^{32}P by random primer labeling (kit available from Stratagene, La Jolla, CA). The 546bp SmaI fragment from SEQ ID NO:1 was hybridized to EcoR1 and KpnI digests of numerous pneumococcal strains on Southern blots. This same fragment was also used to screen a library of Sau3A fragments of genomic DNA from S. pneumoniae strain CP1200.

A 3.5 kb insert was identified from the CP1200 library. The insert was sequenced and an open reading frame of 492 base pairs, including the stop codon, was identified. The open reading frame coded for a polypeptide of 168 amino acids and a predicted molecular weight of about 18,500 daltons.

PCR primers were constructed to amplify the open reading frame; the 5' PCR primer incorporated a *BamHI* site; the 3' primer incorporated a *PstI* site. The amplified insert was ligated in frame to a His-Tagged *E. coli* expression vector pQE30 (Qiagen, San Diego, CA). The resulting plasmid was used to transform *E. coli* strain BL21 (Novagen, Madison, WI) containing the lac repressor plasmid pREP4 (Qiagen). *E. coli* cultures were induced to express the His-Tagged polypeptide and the polypeptide was column purified with Ni-NTA resin (Qiagen). The purified polypeptide was confirmed by SDS-PAGE gel.

Example 2

Identification of a 20kDa C3-Degrading Polypeptide

To determine the C3-degrading capability of the 20 kDa polypeptide, 0.5 mg/ml of C3 (prepared according to Tack et al., *Meth. Enzymol.* 80:64-101, 1984) was copolymerized in a sodium dodecyl sulfate (SDS) gel-containing 15% acrylamide (15% SDS-PAGE gel). Pneumococcal supernatants were obtained from cultures of S. pneumoniae strain CP1200 grown to exponential phase in Todd Hewitt broth; pneumococcal lysates were obtained by incubating 5×10^8 cells with 5% SDS for 30 minutes at room temperature. The lysate was concentrated 10 fold using a Centricon filtration device with a 10,000 mw cutoff (Amicon, Beverly, MA). The samples were not heated before electrophoresis.

Samples of supernatants and lysates were added to the 15% C3-containing SDS-PAGE gels and electrophoresis was carried out at 4°C at 150 V until the dye front ran out. The gel was washed successively with 50 ml of 2.5% Triton X-100 in water (2 times, 10 minutes), 2.5 % Triton X-100 in 50 mM Tris-HCl, pH 7.4 (2 times, 10 minutes), and 50 mm Tris-HCl, pH 7.4 (2 times, 10 minutes) to remove SDS. After washes, 50 ml of 50 mM Tris-HCl, pH 7.4, was poured into dishes containing the gels, and the dishes were covered and incubated at 37°C for 1.5 hour and overnight (about 16 hours). The gels were stained with Coomassie blue for 10 minutes and destained totally.

Two lytic bands were visualized, one of which was about 20 kDa in size, against the dark blue background in both lysates and supernatant. C3 degrading activities in the pneumococcal lysates were observed after a 1.5 hour incubation at 37°C, while C3 degrading activities in the Pn supernatant were observed after an overnight incubation. Therefore, C3 degrading activities appeared to be mainly cell associated.

Example 3

The gene encoding the 20 kD polypeptide is conserved in a number of *S. pneumoniae* strains.

DNA was obtained from a variety of *S. pneumoniae* strains (Clinical isolates of Type 1, Type 3, LOO2 and LOO3 (type 3), Type 4, Type 14 and Laboratory isolates CP1200, WU2, R6X, 6303,109,110, JY1119, JY182, and JY53) and SEQ ID NO:3 was used as a probe to detect the presence of nucleic acid encoding the 20 kD polypeptide in DNA from these strains. Isolated chromosomal DNA was digested with *EcoRI* and separated by electrophoresis. The DNA was transferred to a solid support and hybridized to end-labeled SEQ ID NO:3 under the hybridization and washing conditions of 6X SSC, 5X Denhart's, 0.5% SDS, 100 µg/ml denatured fragmented salmon sperm DNA hybridized at 65 °C overnight and washed in 2X SSC, 1 time at room temperature for 10 minutes and in 2X SSC, 0.1% SDS 1 time at 65°C for 15 minutes followed by two washes in 0.2X SSC, 0.1% SDS for 3 minutes each at room temperature.

Results indicated that SEQ ID NO:3 hybridized identically in each of the DNA samples tested indicating that the polypeptide appears to be conserved among strains. In some strains, the DNA encoding the 20 kDa C3-degrading polypeptide appears to be part of a larger open reading frame of 2478 bp that putatively encodes a 92 kDa polypeptide.

Example 4

Southern blot of *S. pneumoniae* DNA probe

Five ug samples of genomic DNA were obtained from 11 strains *of S. pneumoniae*. Each sample was digested with the restriction enzyme Kpn1. The samples were subsequently loaded onto an agarose gel and resolved by electrophoresis. The samples contained in the gel were subsequently transferred to a Hybond-N+ membrane available from Amersham (Upsalla, Sweden) by capillary transfer. A 540 bp SmaI fragment from the open reading frame was random primer labeled with P^{32} using a ^{T7}QuickPrime kit (Pharmacia, Piscathaway, NJ) and purified from non-incorporated nucleotides using NucTrap column (Stragene, La Jolla, CA) and hybridized.

The hybridization conditions were 6XSSC, 5X Denhardt, 0.5% SDS, and 100 µg/ml fragmented and denatured salmon sperm DNA hybridized overnight at 65°C and washed in 2X SSC, 0.1% SDS once at room temperature for about 10 minutes followed by 1 time at, 65°C for about 15 minutes followed by at least one wash in 0.2XSSC, 0.1% SDS at room temperature for at least 3-5 minutes. The blot demonstrated that the 20 kDa gene was present in all tested strains of *S. pneumoniae*.

Example 5

Two DNA primers were prepared from SEQ ID NO:1 and utilized to amplify the nucleotide sequence encoding the 20 kDa polypeptide from *S. pneumoniae* (serotype 3) genomic DNA. The first primer, a 5'-primer, SEQ ID NO:6, includes an ATG start codon of the 5' end of the nucleotide sequence, inserts a Nco1 site, and had an Ala residue inserted after the ATG start codon to maintain a correct reading frame. The second primer, a 3'-primer, SEQ ID

NO:7, includes a termination codon at the 3' end of the nucleotide sequence and inserts a BamH1 site.

5'-GGGGG CCA TGG CC TCA AGC CTT TTA CGT GAA TTG-3';
(SEQ ID NO:6)

5'-GGGGG GGA TCC CTA GCT ATA TGA GAT AAA CTT TCC TGC T-3'; (SEQ ID NO:7)

The two primers were synthesized on an Applied Biosystems 380A DNA synthesizer (Foster City, CA) using reagents purchased from Glen Research (Sterling, VA). Amplifications were performed utilizing a Perkin Elmer Thermocycler (ABI) according to the manufacturer's directions. The identified PCR product was ligated into the TA tailed PCR cloning vector PCR2.1, available from Invitrogen, Carlsbad, CA, and used to transform OneShot Top10F' competent cells (Invitrogen). Kanamycin resistant transformants were screened by restriction enzyme analysis of plasmid DNA prepared by alkaline lysis. An approximately 500 bp insert fragment was identified and subsequently excised with restriction enzymes Nco1 and BamH1. The 500 bp fragment was purified from a low melting agarose gel, and subsequently ligated into the Nco1-BamH1 sites of the T7 promoted expression vector pET 28a, available from Novagen (Madison, WI).

The ligation mixture was subsequently transformed into Top10F'cells (Invitrogen), and the kanamycin resistant transformants were screened by restriction enzyme analysis of plasmid DNA prepared by alkaline lysis. A recombinant plasmid (pLP505) was subsequently tranformed into BL21 (Novagen) cells and grown in SOB media supplemented with 30 μg/ml kanamycin. Cells were grown to an $O.D._{600}$ of 0.6, and were subsequently induced with 0.4mM IPTG (Boehringer Mannheim, Indianapolis, Indiana) for 2-4 hours. Whole cell lysates were prepared and electrophoresed on a 14% SDS-PAGE gel. The gel was stained with Coomassie and the expression product was detected. The coomassie stained gel revealed a band between the 28 kDa and the

18 kDa molecular weight markers, and was determined to be approximately 20 kDa.

The DNA sequence of the insert in the recombinant pLP505 plasmid was obtained using the ABI 370A DNA sequencer. The DNA sequence was aligned with the DNA sequence of SEQ ID NO:1, using the Pustell DNA matrix plot feature of MacVector (Oxford Molecular Group, Campbell, CA). Alignment of the DNA sequence obtained from the pLP505 plasmid, SEQ ID NO:1, and the *S. pneumoniae* (serotype 4) genome, revealed that the open reading frame (ORF) that codes for the 20 kDa polypeptide may be part of a larger ORF, i.e., a 2478 bp in the serotype 4 genome, that codes for a polypeptide with a predicted MW of approximately 92 kDa (SEQ ID NO: 4). DNA SEQ ID NO:4 encodes for a predicted amino acid sequence as shown in SEQ ID NO:5.

The *S. pneumoniae* (serotype 4) genome sequence was obtained from The Institute for Genomic Research at www.tigr.org and/or through NCBI at www.ncbi.nlm.nih.gov, using the ClustalW feature of MacVector, (Oxford Molecular Group, Campbell CA). A sequence comparison was made between the 20 kDa amino acid sequence (SEQ ID NO:2) and the predicted 92 kDa amino acid sequence (SEQ ID NO:5).

Based upon the available genomic DNA (serotype 4) sequence, two primers flanking the 2478 bp ORF were designed and subsequently synthesized using the ABI 380A DNA synthesizer (SEQ ID NOS:8 and 9). SEQ ID NO:8 was an *S. pneumoniae* 5'-primer having an inserted Nco1 site and a "Glu" residue added after the ATG start codon to maintain a correct reading frame. SEQ ID NO:9, was an *S. pneumoniae* 3'-primer having an inserted HindIII site.

5'- CCC GGG CCA TGG CTA AAA TTA ATA AAA AAT ATC TAG -3'; (SEQ ID NO:8)

5'-CCG GGC AAG CTT TTA CTT ACT CTC CT-3'-; (SEQ ID NO:9)

An approximately 2400 bp DNA fragment was then amplified from the 4 different S. pneumoniae serotypes (serotype 3, 5, 6B and 7) resulting in 4

fragments. Each of the 4 fragments were subsequently ligated into the PCR cloning vector PCR2.1 (Invitrogen), and used to transform OneShot Top 10F' cells (Invitrogen). Kanamycin resistant transformants were screened by restriction analysis of the plasmid DNA prepared by alkaline lysis. A recombinant plasmid containing the serotype 7 PCR product was identified, e.g., pLP512. The DNA sequence was obtained from the serotype 7 clone using the ABI model 370A DNA sequencer. The DNA sequence was essentially identical to SEQ ID NO:4 and encoded a predicted amino acid sequence essentially identical to SEQ ID NO:5.

Example 6
Western blot detection of 92 kDa Polypeptide in Whole Pneumococci

Recombinant approximately 20 kDa C3 degrading polypeptide was purified from *Escherichia coli* strain BLR containing plasmid pDF122. Plasmid pDF122 contains the polynucleotide shown in SEQ ID NO:1 expressed under control of the T7 phage promoter system. The bacterial cells were grown to mid-log phase in Hy-Soy/yeast Extract medium containing ampicillin to select for the plasmid. Expression of the recombinant polypeptide was induced by adding IPTG to a concentration of 1mM and continuing incubation for an additional 3 hours. The bacterial cells were harvested by centrifugation and resuspended in Tris buffered saline, pH 7.2. Cells were mechanically lysed in a French Pressure cell and the insoluble material including inclusion bodies were pelleted in a centrifuge. The pellet was solubilized in 3 M Urea buffered with 100mM $NaPO_4$, pH 8.0 containing 0.1% Triton X-100. After pelleting and discarding insoluble material (centrifuged at ~100,000 x g), the soluble r 20kDa polypeptide was exchanged into 0.1% Zwittergent 3-12 (Calbiochem-Behrng) replacing the Urea and then into 100 mM $NaPO_4$ pH 8.0 replacing the detergent. SDS-PAGE analysis confirmed that the ~20 kDa material remained soluble. The His-tagged recombinant polypeptide was dialyzed into 50 mM $NaPO_4$ buffer, pH 8.0 and absorbed onto a Ni column equilibrated with the same buffer. The column was sequentially washed with 50 mM $NaPO_4$ at pH 7.0, 6.6, and 5.5 until baseline absorbances (OD_{280}) were reached with each buffer. The bound ~r20 kDa

polypeptide was eluted with 50 mM $NaPO_4$, pH 4.5. The eluted polypeptide was approximately 90% homogeneous in SDS-PAGE analysis.

This polypeptide was used to immunize Swiss-Webster mice. Five μg doses of the polypeptide were mixed with 50 μg of Monophosphoryl lipid A (Ribi Immunochemicals) and injected intramuscularly into mice. Animals were immunized at weeks 0, 4, and 6 and exsangruinated at week 8. The sera were pooled together and found to contain high titered antisera against the immunogen.

Pneumococcal strains CP1200, T3, and T7 were grown in Todd-Hewitt broth containing yeast Extract and the cells pelleted by centrifugation. Pneumococcal cells were lysed in SDS-PAGE cracking buffer under reducing conditions by boiling for 5minutes. The lysates were loaded onto a 10% SDS-PAGE gel and electrophoresed. Separated polypeptides were electroblotted onto Nitrocellulose and the filter blocked with 5% BLOTTO in phosphate buffered saline, pH 7.4. The polyclonal antisera was diluted 1:2000 in BLOTTO and used to probe the separated polypeptides. Bound antibodies were detected with Alkaline phosphatase conjugated goat anti-mouse IgG. The Western Blot is shown in Figure 8 and described further in the brief description of the figures.

Example 7

Evidence of that the 20 kDa and 92 kDa Polypeptides Degrade C3

The gel shown in Figure 9 is a Western blot. This data were obtained by incubating a 20 kDa polypeptide and a 92 kDa polypeptide with biotinylated and methylamine-treated C3 as shown in the table below. These polypeptides were expressed from a T7 vector.

METHYLAMINE-TREATED C3: 500 μl of purified human C3 at concentration of 4.46 mg/ml (prepared according to the method of Tack et al., Meth. Enzymol. 80:64-101, 1984) was incubated with 55 μl of 1 M methylamine (CH_3NH_2) in 0.1 M TRIS/0.01 M EDTA, pH 8.0 for 80 minutes at 37°C, then dialyzed overnight in 0.1 M TRIS/0.01 M EDTA, pH 8.0, at 4°C. The following day, the C3 was incubated again with 60 μl of 1 M methylamine in 0.1 M

TRIS/0.01 M EDTA for 80 minutes at 37°C, then dialyzed overnight in 50 mM $NaHCO_3$ at 4°C.

BIOTINYLATION of METHYLAMINE-TREATED C3: 2 mg of methylamine-treated C3, prepared above, was incubated with 75 µl of NSC-biotin (Pierce) made as 1 mg NSC biotin in 1 ml H_20 for 30 minutes at room temperature. The biotinylated C3 was dialyzed overnight in 50 mM $NaHCO_3$, pH 8.0 at 4 °C.

Reagents	Tube A – 20 kDa	Tube C – 92 kDa
Polypeptide	34 µl 20 kDa polypeptide 5.6×10^{-9} moles	100 µl 92 kDa polypeptide 5.6×10^{-9} moles
Biotinylated C3	3 µl C3 71×10^{-12} moles	3 µl C3 71×10^{-12} moles
Buffer	63 µl 0.01% BSA/PBS	63 µl 0.01% BSA/PBS

Samples from Tubes A and C were removed after 66 hours of incubation, reduced by boiling for 2 minutes in reducing buffer with β mercaptoethanol, electrophoresed on 7.5% SDS-PAGE, and transferred to nitrocellulose paper for Western blotting. Western blotting was carried out for one hour according to standard procedures (Tobin et al. *PNAS USA* 76:4350-4354, 1979). Membranes were incubated with horseradish-peroxidase-avidin (1:10,000 dilution) and developed with the Supersignal CL-HRP Substrate System (Amersham) to detect biotinylated C3 fragments.

In Figure 9 lane 1 is C3 alone (control), lanes 2 and 3 are the 20 and 92 kDa polypeptides, and lanes 4 and 5 are again C3 alone (controls). Along the right margin the position of the α-chain of C3, the β-chain of C3, and the C3 fragment present in the control samples are shown. The position of a C3 fragment in the 20 kDa digest and several fragments in the 92 kDa digest are identified.

The second experiment used the reagents of the first (expressed polypeptides, biotinylated C3, and buffer) in the same proportions as shown in

the table above. C3 control samples were removed at time 0, 2 hours, 8 hours, and 24 hours. However, instead of doing a Western blot, the samples were reduced on 7.5% SDS-PAGE, reasoning that some additional degradation fragments might not be biotinylated and would therefore show up on a Coomassie stained SDS-PAGE gel. Indeed, that is what was observed. Only the 20 (lane A8) and 92 (lane C8) kDa polypeptides were analyzed on this gel. The gel of Figure 10 shows along the right margin the position of the α-chain of C3 and the β-chain of C3, and the 92 kDa polypeptide in lane C8. In lane C8 are at least two fragments ranging in size from ~90 kDa to 75 kDa. There are also several fragments beneath the β-chain at 75 kDa. In lane A8 is a major degradation fragment just below the β-chain of C3. Thus, both the 92 kDa polypeptide in lane C8 and the 20 kDa polypeptide in lane A8 degrade C3.

Example 8

Preparation of recombinant 92 kDa protein and generation of poyclonal anitsera

The insert in plasmid pLP512 (see Example 5) was excised with Nco1 and HindIII. A fragment of the expected size was purified from low melting point agarose, and subsequently ligated into the Nco1- HindIII sites of the T7 promoted expression vector pET28a (Novagen, Madison, WI).

The ligation mixture was subsequently transformed into Top10F' cells (Invitrogen) and the kanamycin resistant transformants were screened as described previously in Example 5. A recombinant plasmid (pLP515) was subsequently transformed into BL21 cells (Novagen) and grown in SOB media supplemented with 30 μg/ml kanamycin. Cells were grown to an O.D. $_{600}$ of 0.6, and were subsequently induced with 0.4 mM IPTG (Boehringer Mannheim, Indianapolis, IN) for 2-4 hours. Whole cell lysates were prepared and electrophoresed on a 10% SDS-PAGE gel. Coomassie staining of the gel revealed a new band of approximately 80 kDa as compared to a pre-induction sample.

Recombinant protein coded by the ~92 kDa ORF was purified from *E. coli* strain BL21 (Novagen) containing the plasmid pLP515. Bacterial cells were grown to mid log phase in SOB medium containing 50 μg/ml kanamycin to select for the plasmid. Expresssion of the recombinant polypeptide was induced by addition IPTG to 0.4 mM and continuing incubation for 3 hours. The bacterial cells were harvested by centrifugation and resuspended in Tris-Buffered Saline, pH7.2. Cells were mechanically lysed in a French Pressure Cell and the insoluble material including inclusion bodies were pelleted in a centrifuge at 7700 x g at 4 degrees for 10 minutes. The pellet containing the inclusion bodies was resolubilized and the soluble protein was purified by ion exchange chromatography. Recombinant protein was used to generate polyclonal antibodies in mice. Briefly, 5 μg of protein was adjuvanted for each dose with 20 μg QS21 and injected subcutaneously into the necks of a 6-8 weeks Swiss Webster mice. The mice were bled and vaccinated at week 0, vaccinated at week 4, then bled and exsanguinated at week 6. 10 mice were vaccinated with the recombinant 92 kDa protein adjuvanted with QS21. Pooled sera were used at a 1:1000 dilution to examine whole cell lysates and concentrated culture supernatants from several serotypes of *S. pneumoniae* on a Western Blot. The sera reacted specifically to a protein in whole cell lysates and concentrated culture supernatants whose molecular weight was approximately 90 kDa.

Example 9

The chart of Figure 11 summarizes the results from an intranasal (IN) challenge of CBA/CAHN *xid*/J mice vaccinated with r92 kDa protein, prepared as described in Example 8 (SEQ ID NO:5) adjuvanted with monophosphoryl lipid A (MPL).

10 mice per group were vaccinated subcutaneously in the neck region at weeks 0, 3,and 5, with either 5 μg each of 92 kDa adjuvanted with 50 μg MPL, 1 μg each of Type 3 capsule conjugated to the protein carrier CRM197 adjuvanted with 100 μg aluminum phosphate, or phosphate buffered saline (PBS) alone, in a sample volume of 10 μl. CRM 197 is a genetically detoxified version of diptheria toxin. Each mouse was challenged in at week 7 with 1 x 10^6

cfu's of Type 3 *S. pneumoniae* in a 10 µl sample volume. Nasal tissue was isolated 3 days after challenge and the number of type 3 *S. pneumoniae* colony-forming units per gram of nasal tissue determined by plating on selective media. Type 3 is capsule isolated from S. *pneumoniae* serotype 3, and then conjugated to protein carrier CRM 197, through reductive animation (see U.S. Patent No. 5, 360,897 for preparation of the Type 3 control; see U.S. Patent No. 5,614,382 for genetically detoxified version of ditheria toxin; see U.S.Patent 4,902,506 with regard to using CRM 197 as a carrier).

The chart in Figure 11 shows that in this model, both the negative controls, mice adjuvanted with MPL and näive mice at 6 weeks of age, had a survival rate of approximately 30% when challenged intranasally, while the positive control, the Type 3 conjugate adjuvanted with aluminum phosphate, offered 100% protection against death to the end of the study, approximately 14 days. When mice immunized with r92 kDa were challenged, 100% survived to the end of the study, indicating that r92 kDa protein does offer protection against death from intranasal challenge by Type 3 *S. pneumoniae*.

Example 10

METHODS

Recombinant 92 kDa (r92 kDa) polypeptide (SEQ ID NO:5) were incubated with purified human C3 for 2 hours, 6 hours, and 26 hours at 37°C in the following ratios:

Tube A (Control): C3 - 3µl [7.2x10-12 moles] PBS/0.01% bovine serum albumin (BSA) - 100 µl; Tube B: r92 kDa - 50 µl [5.6x10-10 moles] C3 - 3 µl [7.2x10-12 moles] PBS/0.01% BSA - 50 µl.

At each time point (2, 6, and 26 hours), 20 µl samples were removed from Tubes A and B reducing buffer was added, and the samples were boiled at 100°C for 2 minutes.

After boiling, samples were electrophoresed on 7.5% SDS-PAGE under reducing conditions. The SDS-PAGE gel was stained with Coomassie blue. The resultant gel is shown in Figure 12.

Lane 1 - molecular weight standards (reading from top of gel: 200 kDa, 116 kDa, 97 kDa, 66 kDa, 45 kDa); Lanes 2 and 4 - 2 hour incubation; Lane 2 - C3 control (Tube A); Lane 4 – r92 kDa sample (Tube B); Lanes 5 and 7 - 6 hour incubation; Lane 5 - C3 control (Tube A); Lane 7 – r92 kDa sample (Tube B); Lanes 8 and 10 - 26 hour incubation; Lane 8 - C3 control (Tube A); Lane 10 – r92 kDa sample (Tube B).

INTERPRETATION of GEL

The α-chain of C3 runs at about 115 kDa. The β-chain of C3 runs at about 75 kDa. These are marked on the gel. The band at about 66 kDa is albumin. Lanes 2 and 4 are the 2-hour incubation. Compared to Lane 2 (C3 control), Lane 4 shows cleavage of the C3 α-chain - new fragment at ~97 kDa. Lanes 5 and 7 are the 6-hour incubation. There is the same new 97 kDa cleavage fragment in Lane 7. Lanes 8 and 10 are the 26-hour incubation. Compared to Lane 8 (C3 control), Lane 10 shows continued cleavage of the α-chain.

It will be appreciated by those skilled in the art that while the invention has been described above in connection with particular embodiments and examples, the invention is not necessarily so limited and that numerous other embodiments, examples, uses, modifications and departures from the embodiments, examples and uses may be made without departing from the inventive scope of this application.

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence; wherein the nucleic acid sequence encoding the amino acid sequence of the isolated polypeptide hybridizes under highly stringent hybridization conditions to at least a portion of either (a) the nucleotides in SEQ ID NO:1 or a complementary strand thereof, or (b) the nucleotides in SEQ ID NO:4 or a complementary strand thereof.

2. The polypeptide of claim 1 which is isolated from *S. pneumoniae*.

3. The polypeptide of claim 1 which is a recombinant polypeptide.

4. The polypeptide of claim 1 having a molecular weight of about 15 kDa to about 25 kDa.

5. The polypeptide of claim 1 having a molecular weight of about 75 kDa to about 95 kDa.

6. The polypeptide of claim 1 which degrades human complement protein C3.

7. The polypeptide of claim 1 comprising at least 15 sequential amino acids.

8. An isolated polypeptide comprising at least 15 sequential amino acids of SEQ ID NO:2 or SEQ ID NO:5.

9. An isolated polypeptide comprising SEQ ID NO:2 or SEQ ID NO:5.

10. An immune system stimulating composition comprising an effective amount of the polypeptide of claim 1 and a therapeutically acceptable carrier.

11. The immune system stimulating composition of claim 10 wherein the polypeptide is isolated from *S. pneumoniae.*

12. The immune system stimulating composition of claim 11 further comprising at least one other immune system stimulating polypeptide isolated from *S. pneumoniae.*

13. The immune system stimulating composition of claim 10 wherein the polypeptide is effective to immunize or treat a mammalian subject against *S. pneumoniae* infection or colonization.

14. The immune system stimulating composition of claim 13 wherein the polypeptide is provided in an amount effective to provide a therapeutic effect to the mammalian subject.

15. An antibody capable of binding to the polypeptide of claim 1.

16. The antibody of claim 15 which is a monoclonal antibody.

17. The antibody of claim 16 which is obtained from a mouse, a rat, a goat, a chicken, a human, or a rabbit.

18. An isolated nucleic acid molecule which hybridizes under highly stringent hybridization conditions to at least a portion of either (a) the nucleotides in SEQ ID NO:1 or a complementary strand thereof, or (b) the nucleotides in SEQ ID NO:4 or a complementary strand thereof.

19. The nucleic acid molecule of claim 18 isolated from *S. pneumoniae.*

20. The nucleic acid molecule of claim 19 which encodes a polypeptide that degrades human complement C3.

21. A vector comprising the nucleic acid molecule of claim 18.

22. A cell comprising the nucleic acid of claim 18.

23. The cell of claim 22 which is a bacterium or a eukaryotic cell.

24. An isolated nucleic acid molecule comprising the nucleic acid sequence of SEQ ID NO:1 or its complementary strand or SEQ ID NO:4 or its complementary strand.

25. An RNA molecule transcribed by a double-stranded DNA sequence comprising SEQ ID NO:1 or SEQ ID NO:4.

26. A method for producing an immune response to *S. pneumoniae* in a mammal, the method comprising administering to a mammal a composition comprising:
(a) a therapeutically effective amount of the polypeptide of claim 1; and
(b) a pharmaceutically acceptable carrier.

27. The method of claim 26 wherein the immune response is a B cell response, a T cell response, an epithelial cell response, or an endothelial cell response.

28. The method of claim 26 wherein the polypeptide is at least 15 amino acids in length.

29. The method of claim 26 wherein the composition further comprises at least one other immune system stimulating polypeptide from *S. pneumoniae*.

30. A method for inhibiting *S. pneumoniae*-mediated C3 degradation, the method comprising contacting an *S. pneumoniae* bacterium with an antibody capable of binding to the polypeptide of claim 1.

31. A method for inhibiting C3-mediated inflammation and rejection in xenotransplantation, the method comprising expressing on the surface of an organ of an animal used in xenotransplantation the polypeptide of claim 1.

32. An isolated nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.

33. An isolated polypeptide having at least 80% sequence identify to SEQ ID NO:2 or SEQ ID NO:5.

34. An isolated polypeptide of about 15 kDa to about 25 kDa from *S. pneumoniae* that degrades human complement protein C3.

35. An isolated polypeptide of about 75 kDa to about 95 kDa from *S. pneumoniae* that degrades human complement protein C3.

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Figure 1 (SEQ ID NO: 1)

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Figure 2 (SEQ ID NO: 2)

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 M  S  S  L  L  R  E  L  Y  A  K  P  L  S  E  R  H  V  E  S  D  G  L  I  F>

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 D  P  A  Q  I  T  S  R  T  A  N  G  V  A  V  P  H  G  D  H  Y  H  F  I  P>

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 Y  S  Q  L  S  P  L  E  E  K  L  A  A  I  I  P  L  R  Y  R  S  N  H  W  V>

  230        240        250        260        270        280        290        300
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 P  Q  P  A  P  S  N  P  I  D  E  K  L  V  K  E  A  V  R  K  V  G  D  G  Y>

  380        390        400        410        420        430        440        450
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CAGAAACTCCTCTTACCTCAAGGAGCAATATAGGGTCGGTTCCTAGAAAGTCGTCTTTGTCGTCGTCCGTAACTA
 V  F  E  E  N  G  V  P  R  Y  I  P  A  K  D  L  S  A  E  T  A  A  G  I  D>

        460        470        480        490        500
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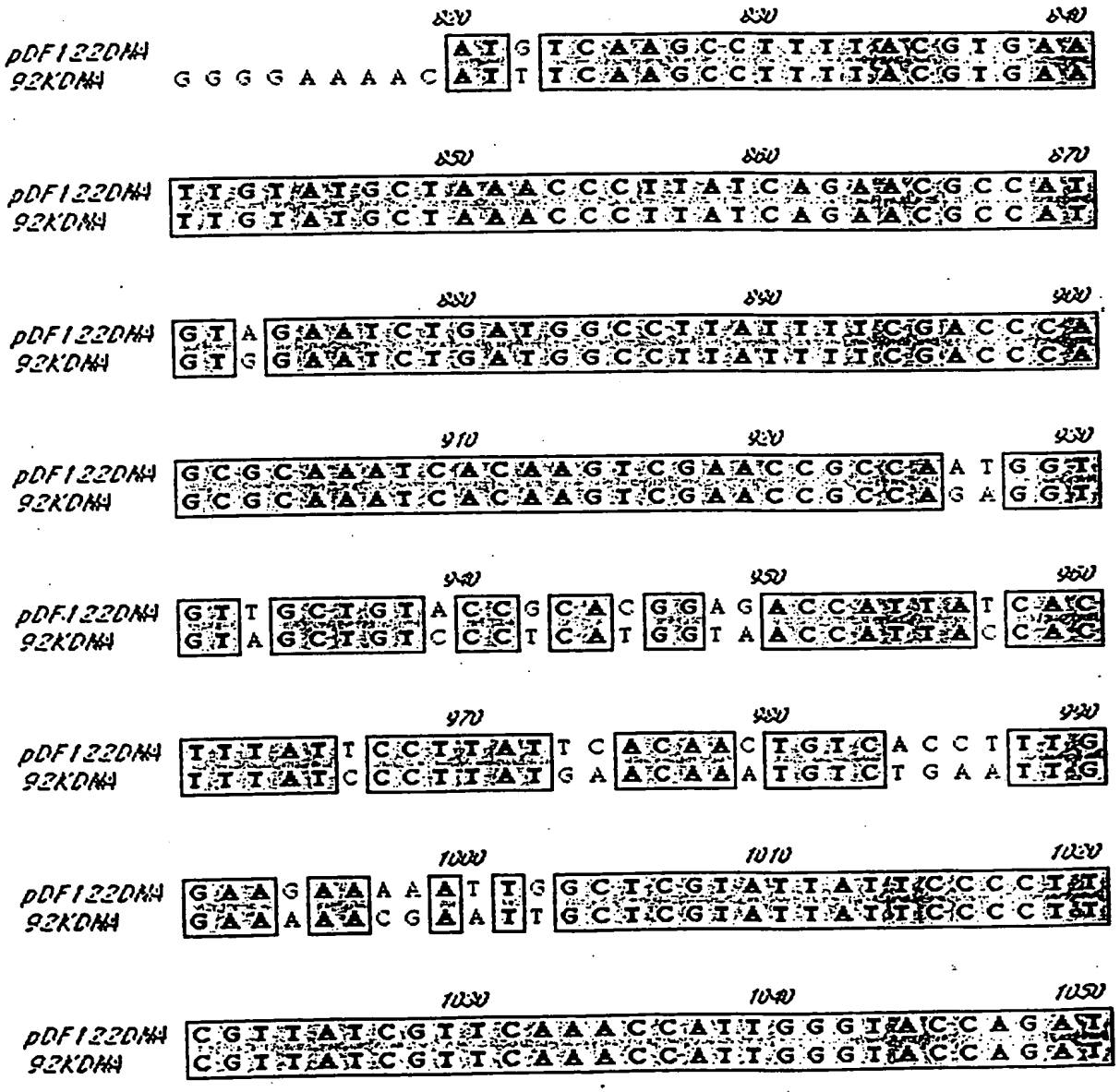
Figure 3
(SEQ ID NOS: 1, 2, and 3)

Fig. 4 (SEQ ID NO:4)

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GAGAGTAAG

Fig. 5 (SEQ ID NO: 5)

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(SEQ ID NOS: 1 and 4)

Fig 6

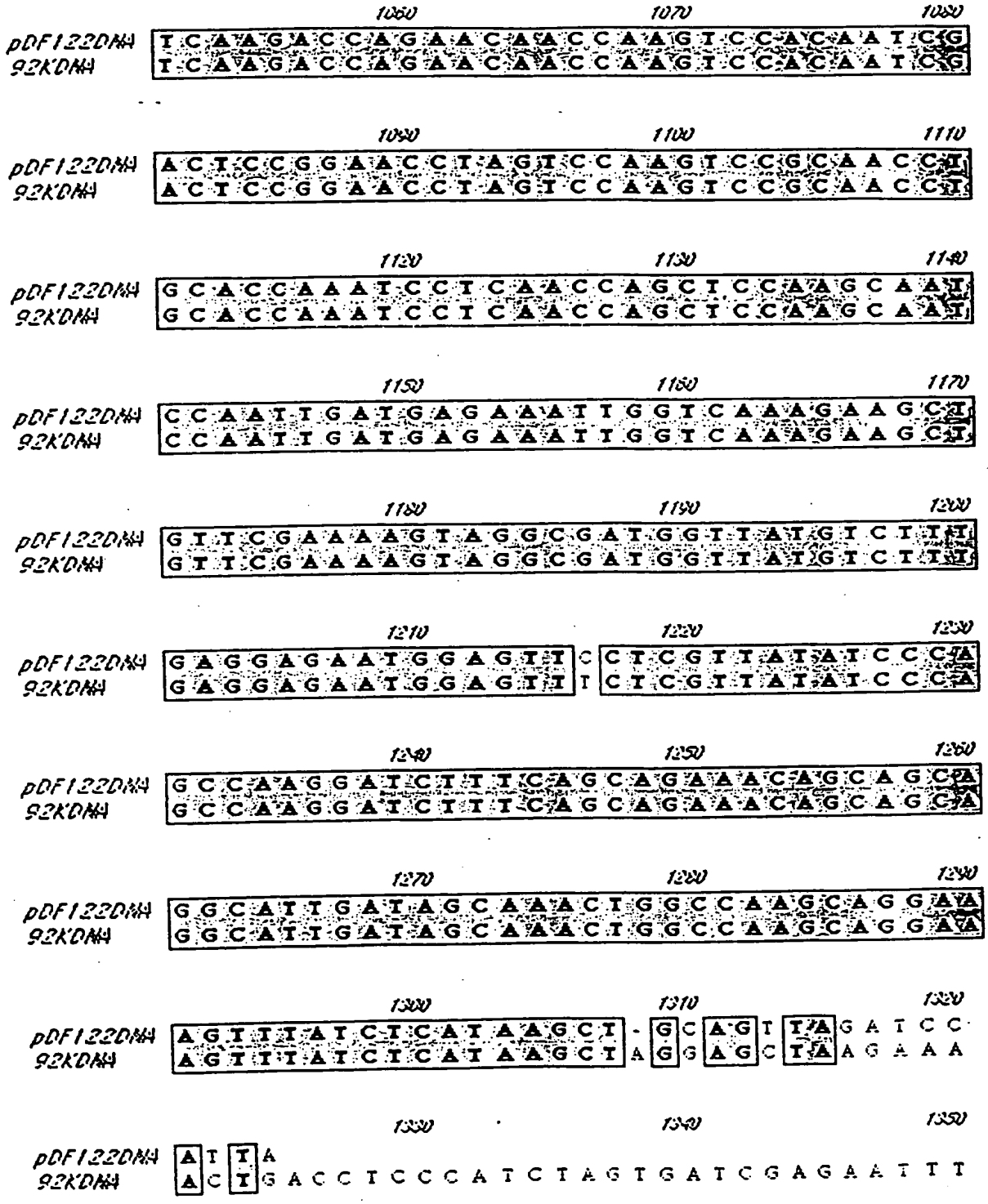


Fig. 6 contd.
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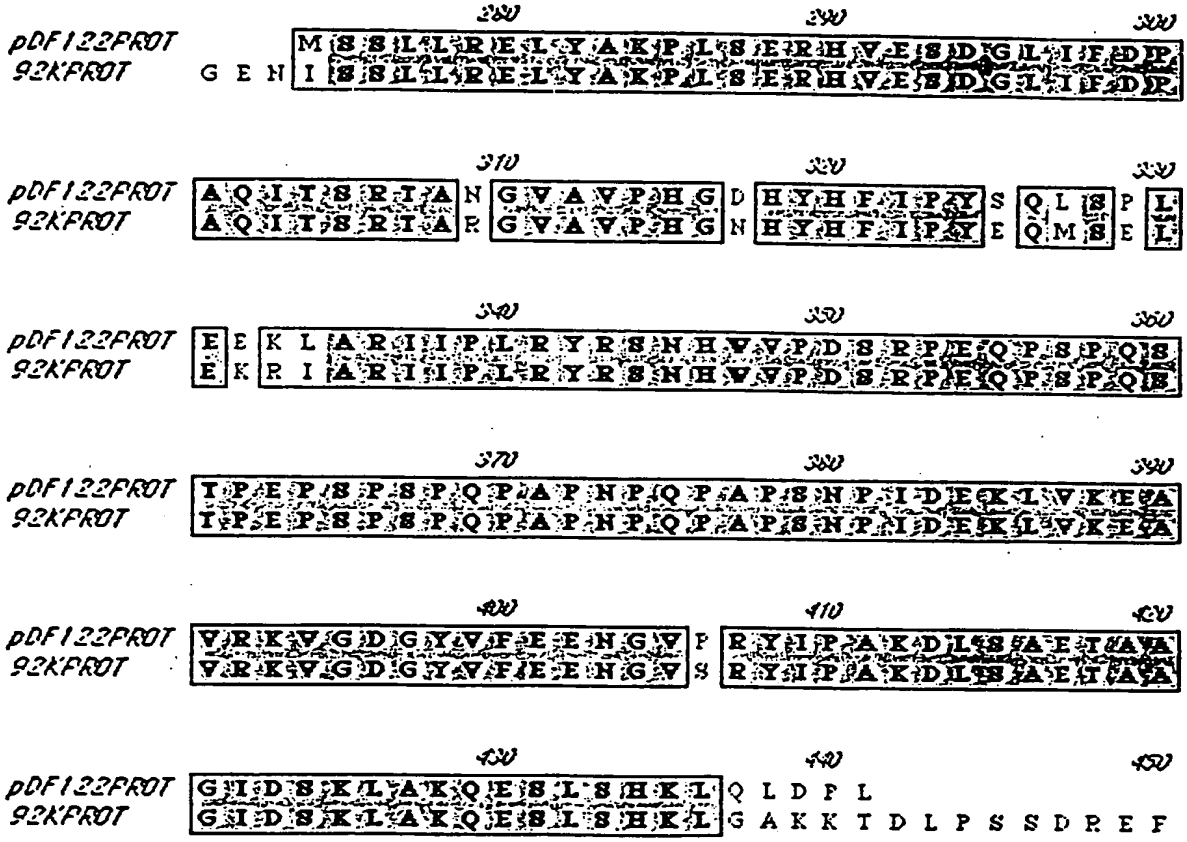


Fig. 7
(SEQ ID NO:2 and a portion of SEQ ID NO:5)

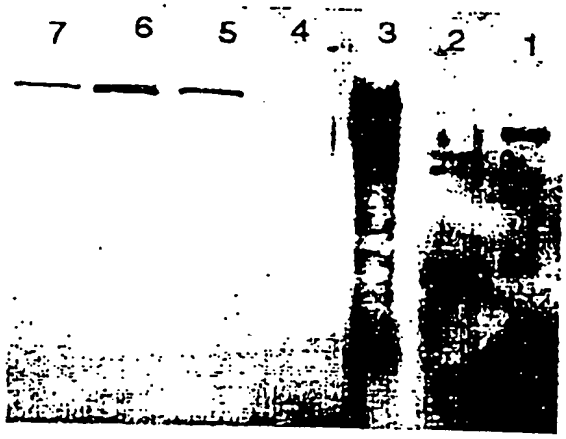


Figure 8

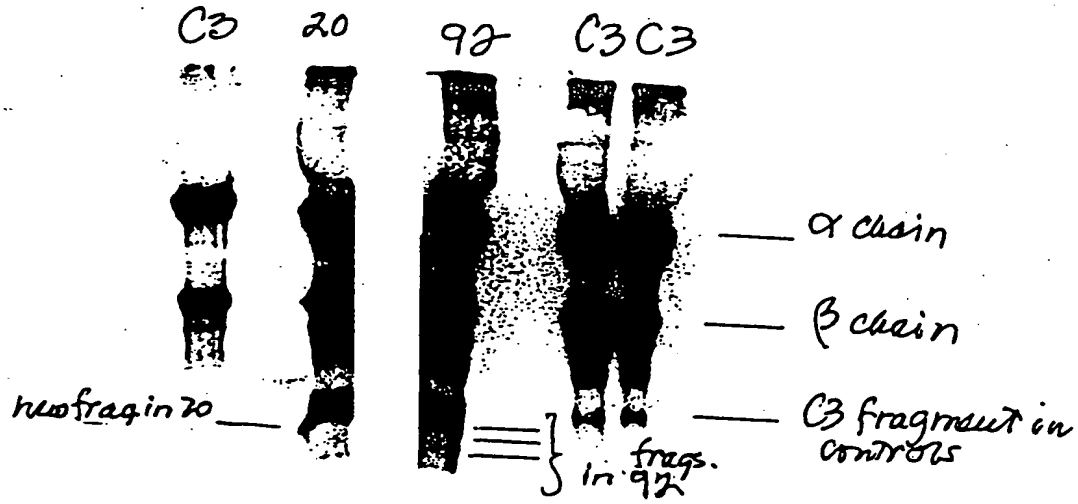

C3
20
92
C3 C3
α chain
β chain
neofragin 20
C3 fragment in controls
frags. in 92

Fig. 9

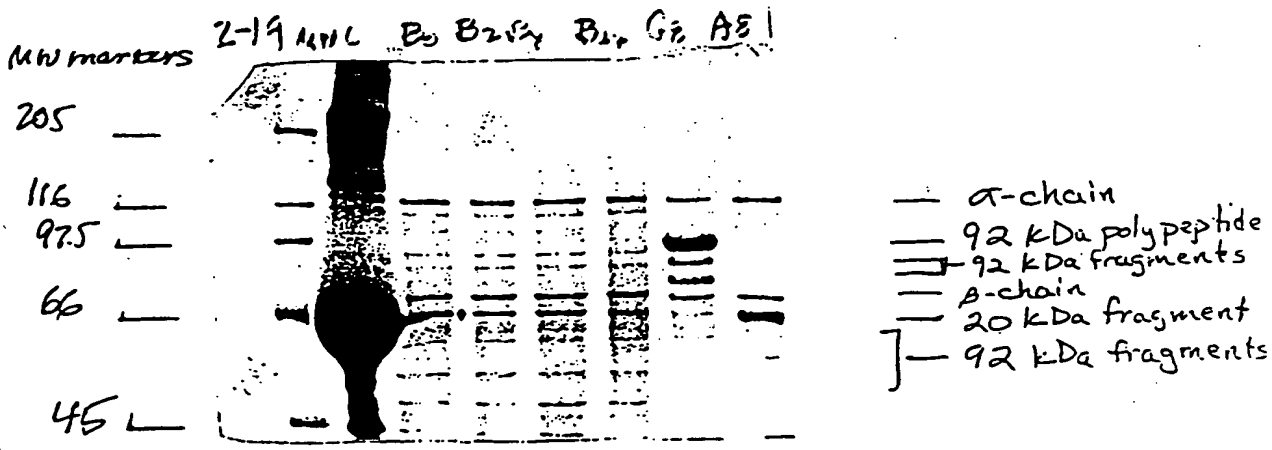
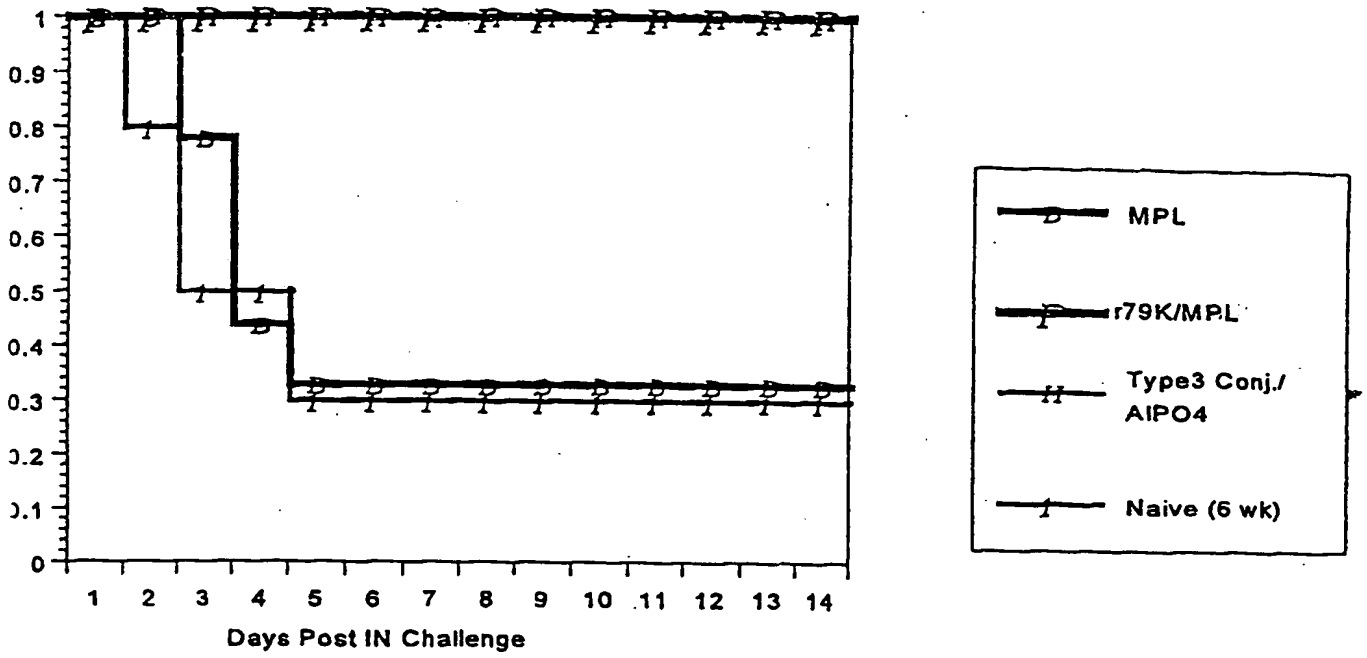

MW markers
205
116
97.5
66
45
α-chain
92 kDa polypeptide
92 kDa fragments
β-chain
20 kDa fragment
92 kDa fragments

Fig. 10



IN = Intranasal

Fig. 11

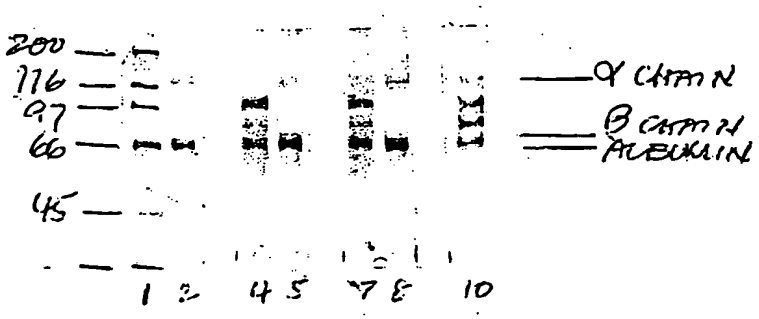


Fig. 12

SEQUENCE LISTING

<110> HOSTETTER, Margaret K.
FINKEL, David J.
CHENG, Qi
MASI, Amy W.
REGENTS OF THE UNIVERSITY OF MINNESOTA
AMERICAN CYNAMID COMPANY

<120> HUMAN COMPLEMENT C3-DEGRADING PROTEINASE FROM STREPTOCOCCUS PNEUMONIAE

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ttggctcgta ttattccccct tcgttatcgt tcaaaccatt gggtaccaga ttcaagacca 240
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cataagctgc agttagatcc atta 504

<210> 2
<211> 168
<212> PRT
<213> Streptococcus pneumoniae

<400> 2
Met Ser Ser Leu Leu Arg Glu Leu Tyr Ala Lys Pro Leu Ser Glu Arg
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His Val Glu Ser Asp Gly Leu Ile Phe Asp Pro Ala Gln Ile Thr Ser
20 25 30

Arg Thr Ala Asn Gly Val Ala Val Pro His Gly Asp His Tyr His Phe
35 40 45

Ile Pro Tyr Ser Gln Leu Ser Pro Leu Glu Glu Lys Leu Ala Arg Ile
50 55 60

Ile Pro Leu Arg Tyr Arg Ser Asn His Trp Val Pro Asp Ser Arg Pro
65 70 75 80

Glu Gln Pro Ser Pro Gln Ser Thr Pro Glu Pro Ser Pro Ser Pro Gln
85 90 95

Pro Ala Pro Asn Pro Gln Pro Ala Pro Ser Asn Pro Ile Asp Glu Lys
100 105 110

Leu Val Lys Glu Ala Val Arg Lys Val Gly Asp Gly Tyr Val Phe Glu
115 120 125

Glu Asn Gly Val Pro Arg Tyr Ile Pro Ala Lys Asp Leu Ser Ala Glu
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Thr Ala Ala Gly Ile Asp Ser Lys Leu Ala Lys Gln Glu Ser Leu Ser
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His Lys Leu Gln Leu Asp Pro Leu
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<210> 3
<211> 504
<212> DNA
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<210> 4
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<212> DNA
<213> Streptococcus pneumoniae

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aatactgagg caaaaggagc agaagctatc tacaaccgcg tgaaagcagc taagaaggtg 1860
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ttattaaagg agagtaag 2478

<210> 5
<211> 826
<212> PRT
<213> Streptococcus pneumoniae

<400> 5

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Lys Lys Glu Ser Asn Arg Val Ser Tyr Ile Asp Gly Asp Gln Ala Gly
        35                  40                  45

Gln Lys Ala Glu Asn Leu Thr Pro Asp Glu Val Ser Lys Arg Glu Gly
    50                  55                  60

Ile Asn Ala Glu Gln Ile Val Ile Lys Ile Thr Asp Gln Gly Tyr Val
65                  70                  75                  80

Thr Ser His Gly Asp His Tyr His Tyr Tyr Asn Gly Lys Val Pro Tyr
                85                  90                  95

Asp Ala Ile Ile Ser Glu Glu Leu Leu Met Lys Asp Pro Asn Tyr Gln
            100                 105                 110

Leu Lys Asp Ser Asp Ile Val Asn Glu Ile Lys Gly Gly Tyr Val Ile
        115                 120                 125

Lys Val Asp Gly Lys Tyr Tyr Val Tyr Leu Lys Asp Ala Ala His Ala
    130                 135                 140

Asp Asn Ile Arg Thr Lys Glu Glu Ile Lys Arg Gln Lys Gln Glu His
145                 150                 155                 160

Ser His Asn His Gly Gly Gly Ser Asn Asp Gln Ala Val Val Ala Ala
            165                 170                 175
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Arg Ala Gln Gly Arg Tyr Thr Thr Asp Asp Gly Tyr Ile Phe Asn Ala
            180                 185                 190

Ser Asp Ile Ile Glu Asp Thr Gly Asp Ala Tyr Ile Val Pro His Gly
        195                 200                 205

Asp His Tyr His Tyr Ile Pro Lys Asn Glu Leu Ser Ala Ser Glu Leu
    210                 215                 220

Ala Ala Ala Glu Ala Tyr Trp Asn Gly Lys Gln Gly Ser Arg Pro Ser
225                 230                 235                 240

Ser Ser Ser Ser Tyr Asn Ala Asn Pro Ala Gln Pro Arg Leu Ser Glu
                245                 250                 255

Asn His Asn Leu Thr Val Thr Pro Thr Tyr His Gln Asn Gln Gly Glu
            260                 265                 270

Asn Ile Ser Ser Leu Leu Arg Glu Leu Tyr Ala Lys Pro Leu Ser Glu
        275                 280                 285

Arg His Val Glu Ser Asp Gly Leu Ile Phe Asp Pro Ala Gln Ile Thr
    290                 295                 300

Ser Arg Thr Ala Arg Gly Val Ala Val Pro His Gly Asn His Tyr His
305                 310                 315                 320

Phe Ile Pro Tyr Glu Gln Met Ser Glu Leu Glu Lys Arg Ile Ala Arg
                325                 330                 335

Ile Ile Pro Leu Arg Tyr Arg Ser Asn His Trp Val Pro Asp Ser Arg
            340                 345                 350

Pro Glu Gln Pro Ser Pro Gln Ser Thr Pro Glu Pro Ser Pro Ser Pro
        355                 360                 365

Gln Pro Ala Pro Asn Pro Gln Pro Ala Pro Ser Asn Pro Ile Asp Glu
    370                 375                 380

Lys Leu Val Lys Glu Ala Val Arg Lys Val Gly Asp Gly Tyr Val Phe
385                 390                 395                 400

Glu Glu Asn Gly Val Ser Arg Tyr Ile Pro Ala Lys Asp Leu Ser Ala
                405                 410                 415

Glu Thr Ala Ala Gly Ile Asp Ser Lys Leu Ala Lys Gln Glu Ser Leu
            420                 425                 430

Ser His Lys Leu Gly Ala Lys Lys Thr Asp Leu Pro Ser Ser Asp Arg
        435                 440                 445

Glu Phe Tyr Asn Lys Ala Tyr Asp Leu Leu Ala Arg Ile His Gln Asp
    450                 455                 460

Leu Leu Asp Asn Lys Gly Arg Gln Val Asp Phe Glu Ala Leu Asp Asn
465                 470                 475                 480

Leu Leu Glu Arg Leu Lys Asp Val Pro Ser Asp Lys Val Lys Leu Val
                485                 490                 495

Asp Asp Ile Leu Ala Phe Leu Ala Pro Ile Arg His Pro Glu Arg Leu
            500                 505                 510
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Gly Lys Pro Asn Ala Gln Ile Thr Tyr Thr Asp Asp Glu Ile Gln Val
515 520 525

Ala Lys Leu Ala Gly Lys Tyr Thr Thr Glu Asp Gly Tyr Ile Phe Asp
530 535 540

Pro Arg Asp Ile Thr Ser Asp Glu Gly Asp Ala Tyr Val Thr Pro His
545 550 555 560

Met Thr His Ser His Trp Ile Lys Lys Asp Ser Leu Ser Glu Ala Glu
565 570 575

Arg Ala Ala Ala Gln Ala Tyr Ala Lys Glu Lys Gly Leu Thr Pro Pro
580 585 590

Ser Thr Asp His Gln Asp Ser Gly Asn Thr Glu Ala Lys Gly Ala Glu
595 600 605

Ala Ile Tyr Asn Arg Val Lys Ala Ala Lys Lys Val Pro Leu Asp Arg
610 615 620

Met Pro Tyr Asn Leu Gln Tyr Thr Val Glu Val Lys Asn Gly Ser Leu
625 630 635 640

Ile Ile Pro His Tyr Asp His Tyr His Asn Ile Lys Phe Glu Trp Phe
645 650 655

Asp Glu Gly Leu Tyr Glu Ala Pro Lys Gly Tyr Thr Leu Glu Asp Leu
660 665 670

Leu Ala Thr Val Lys Tyr Tyr Val Glu His Pro Asn Glu Arg Pro His
675 680 685

Ser Asp Asn Gly Phe Gly Asn Ala Ser Asp His Val Gln Arg Asn Lys
690 695 700

Asn Gly Gln Ala Asp Thr Asn Gln Thr Glu Lys Pro Ser Glu Glu Lys
705 710 715 720

Pro Gln Thr Glu Lys Pro Glu Glu Glu Thr Pro Arg Glu Glu Lys Pro
725 730 735

Gln Ser Glu Lys Pro Glu Ser Pro Lys Pro Thr Glu Glu Pro Glu Glu
740 745 750

Ser Pro Glu Glu Ser Glu Glu Pro Gln Val Glu Thr Glu Lys Val Glu
755 760 765

Glu Lys Leu Arg Glu Ala Glu Asp Leu Leu Gly Lys Ile Gln Asp Pro
770 775 780

Ile Ile Lys Ser Asn Ala Lys Glu Thr Leu Thr Gly Leu Lys Asn Asn
785 790 795 800

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<210> 6
<211> 34

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<220>
<223> Incorporates a NcoI site and a DNA codon for ala.

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<210> 7
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<220>
<223> Incorporates a BamH1 site.

<400> 7
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<210> 8
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<220>
<223> Incorporates a Nco1 site and a DNA codon for Glu.

<400> 8
cccgggccat ggctaaaatt aataaaaaat atctag 36

<210> 9
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<220>
<223> Incoporates a HindD III site.

<400> 9
ccgggcaagc ttttacttac tctcct 26

<210> 10
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Probe

<220>

<223> Oligonucleotide

<400> 10.
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/22302

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/57 C12N9/52 C07K16/40 A01K67/027 A61K38/48
A61P37/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 18930 A (HUMAN GENOME SCIENCES INC ;CHOI GIL H (US); HROMOCKYJ ALEX (US); J) 7 May 1998 (1998-05-07) SEQ ID NOs 55, 56, 65 & 66 page 98; claims 1-21 ---	1-31, 33-35
X	WO 98 18931 A (DOUGHERTY BRIAN A ;HUMAN GENOME SCIENCES INC (US); ROSEN CRAIG A () 7 May 1998 (1998-05-07) SEQ ID NO 94, 243, 258 claims 6-20 --- -/--	1-31, 33-35

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 2000	24.02.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van Klompenburg, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED T BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANGEL ET AL: "Degradation of C3 by Streptococcus pneumoniae" JOURNAL OF INFECTIOUS DISEASES,US,CHICAGO, IL, vol. 170, no. 3, 1 January 1994 (1994-01-01), pages 600-608, XP002092872 ISSN: 0022-1899	34,35
A	page 607, column 1; figures 3,6-8	1-33
X	NANDIWADA L S ET AL: "Genetic analysis of a C3 degrading proteinase in Streptococcus pneumoniae" ABSTRACTS OF THE GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY,US,AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, 1 January 1996 (1996-01-01), page 177 XP002076135 ISSN: 0067-2777	34
A	abstract	1-33,35
A	WO 97 26008 A (UNIV MINNESOTA ;CLEARY PAUL P (US)) 24 July 1997 (1997-07-24) page 3, line 30 -page 5, line 2; claims 1-21; examples 1,4,6	1-35
L,P, X	WO 99 15675 A (GREEN BRUCE A ;CHENG QI (US); FINKEL DAVID J (US); MASI AMY W (US)) 1 April 1999 (1999-04-01) claims 1-61; figures 1-7; examples 1-5	1-35
P,X	WO 98 48022 A (NANDIWADA LAKSHMI S ;DUNNY GARY (US); UNIV MINNESOTA (US); HOSTETT) 29 October 1998 (1998-10-29) page 4, line 6 - line 10; claim 43; examples 1-5	34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/22362

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 26-30 are directed to a method of treatment of the human/animal body, and claim 31 at least partially, the search has been carried out and based on the alleged effects of the compound/composition.

2. [] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[] The additional search fees were accompanied by the applicant's protest.

[] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

ormation on t family members

Interm nal Applicati lo
PCT/US 99/22302

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9818930 A	07-05-1998	AU 5194598 A	22-05-1998
		AU 6909098 A	22-05-1998
		EP 0942983 A	22-09-1999
		EP 0941335 A	15-09-1999
		WO 9818931 A	07-05-1998
WO 9818931 A	07-05-1998	AU 5194598 A	22-05-1998
		AU 6909098 A	22-05-1998
		EP 0942983 A	22-09-1999
		EP 0941335 A	15-09-1999
		WO 9818930 A	07-05-1998
WO 9726008 A	24-07-1997	US 5846547 A	08-12-1998
		AU 705732 B	27-05-1999
		AU 1582897 A	11-08-1997
		CA 2243755 A	24-07-1997
		EP 0877624 A	18-11-1998
WO 9915675 A	01-04-1999	AU 9510598 A	12-04-1999
WO 9848022 A	29-10-1998	AU 7156698 A	13-11-1998